PLANT BIOLOGY TODAY

ADVANCES & CHALLENGES

PLANT BIOLOGY TODAY

ADVANCES AND CHALLENGES

A Symposium Sponsored by the
American Association for the Advancement of Science
and the Botanical Society of America

Edited by
William A. Jensen
Leroy G. Kavaljian

Wadsworth Publishing Company, Inc.
Belmont, California

FOREWORD

This collection of papers was part of a symposium held in December 1961 at the Denver meeting of the American Association for the Advancement of Science. The Botanical Society of America, at the suggestion of its Committee on Education, joined with the Botanical Sciences Section of the AAAS in sponsoring the program.

Clearly, there is a need to make public the information and points of view currently existing in the various fields of research in the plant sciences. It is important to all those interested in the biological sciences—whether they be advanced high-school students, college undergraduate and graduate students, or teachers at all levels—to know the present status of botanical research so that they may be up to date in their facts and concepts and able to see some of the directions in which advances are being made and will be made in the future. Today advances in all of the plant sciences are rapid; and the reports of them, published in many different journals, are becoming increasingly numerous. As a result, unless a person is near a large botanical library, has a good background of information, and a great deal of time to read and to discuss with other knowledgeable people what he has read, it is almost impossible for him even to be aware of advances that are being made—let alone understand them and see where they may lead.

Although these papers present details that may be altered by investigations in the near future, they are nevertheless more current in their particular areas than textbooks usually can be. By noting the trends of thinking and investigation, the reader can feel that he

is now a step ahead of where he was, or at least that he has now been brought abreast somewhat in certain areas and is thus better equipped to keep up in others.

Harriet B. Creighton
Secretary, Botanical Sciences Section, AAAS

PREFACE

Advances. Challenges. These words, which characterize modern science, ring out in virtually every report of current developments in chemistry, physics, and biology. In some ways, these words are most valid for the life sciences. Here the challenges are especially great; for the problems are difficult and profound, and the advances are as exciting as they are hard won. This volume presents a series of papers concerned with problems of immediate importance in the plant sciences—problems that illustrate graphically both the advances and challenges which are the very essence of current biological research.

Five topics are presented: (1) molecular botany, (2) the problem of cell development in plants, (3) photosynthesis, (4) biological clocks, and (5) the movement of dissolved substances within plants. In each paper the writer has attempted to give the reader background information and some knowledge of the most recent advances in the particular field. The problems discussed are currently under intensive investigation, with new data accumulating almost daily.

Each of the authors has worked in some area of the problem he discusses, and each is a recognized research scientist who has added significantly to the area he is investigating. For these reasons a sense of excitement pervades all the papers as the data are discussed and new, unsolved problems are described. The life of the scientist in the laboratory revolves around the ever present unsolved problem. Unfortunately, not all problems are solved nor all questions

answered. Advances are made unevenly, and the research botanist encounters as many stone walls as he does breakthroughs.

An example of a field in a phase of rapid development following a series of brilliant breakthroughs is that of molecular botany, described by James Bonner of the California Institute of Technology. Here discovery follows discovery as data made available from new techniques fall into place with new concepts, building an ever increasing understanding of the molecular control of cell processes. The field of photosynthesis, treated by Lawrence Bogorad of the University of Chicago, is another example of the same type of development. As Dr. Bogorad makes clear, our understanding of photosynthesis is not complete; but advances are being made at a rapid rate, and the basic concepts of the field are being constantly challenged.

A field in a completely different phase of development is discussed by Beatrice Sweeney of Yale University in her paper on biological clocks in plants. The existence of such timekeeping mechanisms in plants and animals has been known for only a short time. Hence, researchers in the area are engaged in exploring the limits of the problem and attempting to formulate basic concepts by which they can proceed. The problem of cell development, presented in the second paper by William Jensen of the University of California at Berkeley, is in a similar stage of research. Here the basic problem is to understand what factors control the development of the plant cells; but before researchers can answer this question, they must first understand the course of cell development. The paper describes attempts to elucidate the changes occurring in cells as they undergo growth and differentiation.

Finally, there are fields that, though progress at present is slow, present a special challenge and require a special type of understanding. Frank Salisbury of Colorado State University presents an example of one of these fields in his paper on the movement of dissolved substances in plants. This is a problem which has interested biologists for centuries and about which much has been discovered. At the same time, a clear understanding of many basic problems of translocation in plants is still beyond our reach and awaits new techniques and concepts.

This collection gives more than just a summary of old information and also more than just the recent advances. It reflects the growth and development of modern botany in the form of dis-

cussions of a variety of important problems. Science can advance only as men recognize and rise to the challenges that exist. We hope that we have made some of these challenges as real and exciting to the reader as they are to us.

William A. Jensen

Leroy G. Kavaljian

CONTENTS

1

MOLECULAR BOTANY

James Bonner

Division of Biology
California Institute of Technology

These days we hear a great deal about molecular biology: that molecular biology is the cutting edge of the biological sciences, that it is slicing off new knowledge of life. However, we hear very little—in fact, almost nothing—about molecular botany.

Molecular botany is that part of botany which comes to grips with the ways in which molecules interact to keep the plant alive. It is concerned with where enzyme molecules come from, what genes are, how genes do their work of causing a whole magnificent plant to be assembled. Unfortunately, since there are only a very few molecular botanists practicing in the world today, we do not yet know all about these matters.

Molecular botany is concerned with the particles and structures

found inside of cells. All cells of all plants, and indeed of all animals too, are very much alike with respect to the kinds of particles and structures which move around inside them. A plant cell—on the average perhaps 100 microns in diameter—is surrounded by a more or less rigid cell wall, which provides the cell with a protective coat. Inside this carbohydrate cell wall is a fatty structure, a membrane. It is the function of this membrane to keep inside the cell all of the things that must remain inside, but at the same time to let in food molecules and water. Within the membrane are structures with which every botanist is acquainted, having seen them through the microscope. These include, first of all, the nucleus. Most plant cells have a nucleus, which is some 10 to 20 microns in diameter. If the cell is a photosynthetic one, it also contains chloroplasts, perhaps fifty in number and 5 to 10 microns in diameter, which are large enough to be seen in the light microscope. With the aid of the light microscope one may also see the numerous mitochondria, which are spherical or rod-shaped particles, 1 or more microns in diameter and perhaps five hundred in number. Since the early 1950's—with the development of electron microscopy, which enables one to see particles much smaller than those visible by light microscopy—botanists have been able to see in plant cells a still smaller type of particle. This particle, some 20 millimicrons in diameter, has been named ribosome. Ribosomes are not only small, they are extremely numerous; the typical plant cell contains about a half million of them. Through the electron microscope one may also distinguish the even smaller enzyme molecules, which are about 2 millimicrons in diameter and in number altogether total perhaps 500 million per cell. Though they are so small that they are as yet invisible by microscopy, plant cells are presumed to contain food molecules such as those of sugars recently photosynthesized, building blocks for the making of enzymes, and substances intermediate between recently synthesized food molecules and enzyme building blocks.

All of these entities—nucleus, chloroplasts, mitochondria, ribosomes, enzymes, food and metabolite molecules—together make up the cytoplasm of the plant cell. The cytoplasm of the plant cell surrounds the central vacuole. This vacuole, composed of water, salts, sometimes tannins, and other small molecules, does not contain cytoplasm, but is merely a watery kind of "waste dump" for the cell.

A principal objective of molecular botany has been to find out what each of these different particles contributes to the life of the cell. The problem is most easily attacked by first separating the different kinds of subcellular objects from one another, in order to discover what each is made of and what each does. One of the great triumphs of molecular botany of the last ten years has been the development of simple methods to accomplish this separation, which is actually quite simple. Some plant cells, a group of cells or a tissue, or even a whole organ such as a root or a stem is ground up, and, by this grinding, tears are made in the cell walls and membranes of each of the cells. The ground tissue is then placed in a cheesecloth, or other readily available kind of bag, and squeezed. The cell-wall particles are sufficiently large so that they stay within the bag, but the juice which is pressed out contains the subcellular particles. The bag now contains a suspension composed of nuclei and all the other things which are inside cells. These entities next must be separated from one another. This is done simply by taking the suspension of subcellular particles and putting it in a centrifuge tube. The centrifuge tube is then spun so that the particles of the suspension are subjected to a sedimenting force greater than one times gravity: $1 \times g$. Under these conditions the largest and heaviest objects in the suspension, the nuclei, sediment first to the bottom of the centrifuge tube.

The upper layer of solution above the sedimented nuclei (or the supernatant, as it is called) can be decanted. The nuclei can be resuspended in water or another appropriate medium. There now exists a pure preparation of nuclei. The supernatant containing chloroplasts and smaller particles can be centrifuged again, using a slightly greater centrifugal force. The second largest and densest particles, the chloroplasts, then sediment to the bottom. To sediment nuclei ordinarily requires a force on the order of 100 to $200 \times g$ for ten or fifteen minutes. To sediment chloroplasts requires 1,000 to $2,000 \times g$ for about fifteen minutes. Once the chloroplasts have been removed, sedimenting the mitochondria requires centrifugation at about $10,000 \times g$ for fifteen minutes. The sedimentation of ribosomes requires still higher centrifugal forces on the order of $100,000 \times g$ for one hour. In principle, the enzyme molecules too could be sedimented out in this same way by centrifugation. However, enzyme molecules are very small and the centrifugal forces required for their sedimentation would be very great.

It has turned out to be easier to separate enzymes by other methods, such as by precipitation with ammonium sulfate or by the application of other chemical methods commonly used in enzymology. It is possible, then, to separate each of the subcellular entities of the plant cell, and to collect representative portions of each in relatively pure form.

We are now in a position to inquire what each type of subcellular particle is made of, and what each does. Let us start with the enzyme molecules. There are, in a typical plant cell, about 10,000 different kinds of enzyme molecules. Since there are about 500 million enzyme molecules altogether in the cell, this means that each cell contains about 50,000 representatives of each of the 10,000 kinds of enzyme molecules. With what are these enzyme molecules concerned? It is a principle of molecular botany, and indeed of all of biology, that for every kind of chemical reaction conducted in a living entity, there is an enzyme molecule whose responsibility it is to speed and catalyze this reaction—to make it go more rapidly than it would in the absence of the enzyme. Plant cells, and indeed all cells, by virtue of the presence of an appropriate enzyme, speed each of the many kinds of chemical reactions which are required to transform available food molecules into the building blocks for making more cellular material. Since there are about 10,000 kinds of enzyme molecules in the typical plant cell, there are correspondingly about 10,000 kinds of enzyme reactions going on in that cell.

Two of the subcellular particles of the plant cell, the mitochondria and the chloroplasts, may be viewed as special cases of enzyme molecules. Functionally, the mitochondria are composed of little packets, each of which contains about fifty different kinds of enzyme molecules, all joined together in a unit. This unit conducts all of the reactions of respiration—the reactions by which food is burned with oxygen to carbon dioxide and water, and in which a portion of the energy released is conserved in forms suitable for use in the energy-using reactions of the cell. The mitochondrion is a pre-packaged group of certain kinds of enzymes appropriate for the conduct of the respiratory reactions. Similarly, the chloroplast is composed of units of a small number of the kinds of enzymes that are required for the conduct of photosynthesis; for the capture of light energy; and, with the aid of the energy thus obtained, for the conversion of carbon dioxide to sugar with the concomitant release of oxygen.

Let us now return to enzymes in general. We must know that each of the 10,000 different kinds of enzymes of the characteristic plant cell is a unique and specific kind of chemical compound, different from all other kinds of enzymes. All enzyme molecules belong to that great group of substances known as protein. In essence, a protein is a long-chain molecule made of building blocks attached together, one after the other. In a typical enzyme molecule this chain is about 150 building blocks long. The building blocks of which enzyme molecules are made are known as amino acid molecules; in the typical enzyme, therefore, about 150 amino acid molecules are joined together to form the enzyme molecule. There are only twenty different kinds of amino acid building blocks in all plant and animal cells. In all enzyme molecules the long chain of 150 amino acid building blocks is made of these twenty different kinds of building blocks. It is the sequence in which the different species of building blocks succeed one another down the length of the chain that determines what kind of enzyme molecule the enzyme is. We might think of it in this way: an enzyme molecule can be considered a message written in the twenty-letter alphabet of the amino acids. In order for an enzyme to have its proper function, each message must have a certain sequential order of letters when the enzyme molecule is being made. Just one wrong building block inserted anywhere along the chain can be disastrous, because the result would be an enzyme molecule which is inactive in carrying out the assigned reaction.

Our next question then, is, where do enzyme molecules come from? Enzyme molecules are not alive. One can take enzyme molecules and put them in a beaker with an assortment of the appropriate kinds of building blocks, but the enzyme molecules will not reproduce themselves. They will remain inert, for they do not have the basic life property, the ability to multiply. On the contrary, enzyme molecules are made. It is the function of the ribosomes to synthesize enzyme molecules. Each ribosome possesses the power of synthesizing one particular kind of enzyme molecule. Since there are 10,000 kinds of enzyme molecules to be made, and since there are 500,000 ribosomes in a typical cell, this means there are about fifty ribosomes for making each of the 10,000 kinds of enzyme molecules of the plant cell. How do the ribosomes do their task? The assemblage of an enzyme molecule is obviously a job that requires considerable information. If we wish to make an enzyme

molecule, we must have the recipe; we must know the order in which to assemble the twenty different kinds of building blocks in the long chain that constitutes the particular kind of enzyme. As molecular botanists now know, the ribosome is a particle containing information. The information about how to make the particular kind of enzyme the ribosome is supposed to make is contained in the ribosome in the form of what we might imagine to be a printed tape. On this long printed tape there is written down, in the form of a series of information units, instructions on how to make the desired enzyme molecule. The tape containing instructions is composed of a series of information units, the first of which says something like, "To read the information which I contain, please start here." (It is important not to start reading at the wrong end and get it all backward.) The second information unit says something like, "To make the enzyme for which I have information, please put amino acid of kind No. X (whichever is the right kind) here." The third information unit may say, "To make this particular kind of enzyme for which I have information, please put amino acid of kind No. Y here"—and so forth. And so the proper kinds of amino acid molecules are assembled along the long tape containing information, and when they are all assembled correctly in place, still another enzyme—one of the 10,000—catalyzes the attachment of all of the amino acid building blocks into a long chain. The enzyme molecule is now fabricated; it leaves the ribosome and can begin to function as a catalyst for the appropriate chemical reaction. The ribosome is free to start upon the assemblage of another molecule from the amino acid building blocks. By the time a molecular botanist comes along and grinds up the plant cell, each ribosome has fabricated something like 1,000 of the enzyme molecules for which it contains information.

The tape of the ribosome containing information is, then, the interesting part of this structure. This tape is made of a specific tape substance, ribonucleic acid (abbreviated RNA). RNA also is a long-chain molecule, a long chain made of four different kinds of building blocks, which succeed one another in any one of many permutations and combinations down the length of the tape. The information of the tape is in the order in which these four kinds of building blocks succeed one another. The tape is a kind of message written in the four-letter alphabet of RNA. It is a message that gives instructions, then, as to the order in which to put

together the twenty different kinds of amino acids in order to assemble a specific kind of enzyme molecule. Since there are twenty different kinds of amino acids to be specified in the four-letter language of nucleic acid, obviously the message units in the tape, which specify the kind of amino acid to be inserted next, must consist of several successive letters of the RNA alphabet. In fact, it is generally held, although it has not been rigorously demonstrated, that the message units of the ribosomal tape are composed of three successive letters, since it requires at least three-letter words in a four-letter language to specify twenty different kinds of things, such as the twenty different kinds of amino acid building blocks. And so we can think of the ribosomal tape as a long message made of a series of three-letter code words, each of which represents one particular kind of amino acid building block. The translation from the four-letter language of RNA to the twenty-letter language of amino acids and therefore of enzymes is known to biologists today as a coding problem. It is a problem that is just beginning to be understood; that is, we are just beginning to be able to translate from RNA language to enzyme language. We know, for example, which three letters are contained in each of the three-letter code words that make up the ribosomal tape; and we know which three RNA letters are contained in each of the twenty code words that represent each of the twenty different kinds of amino acids. However, we do not yet know in detail the sequence in which the three letters succeed one another in the code words, though this knowledge will quite likely be achieved within the next few months or years.

Our next question, then, is where do the ribosomes come from? Ribosomes, like enzyme molecules, are not alive; they do not possess the power to duplicate themselves. Like enzyme molecules, they are manufactured, and, in fact, they are manufactured in the nucleus. This is clear from several different kinds of considerations.

In the first place, cells without nuclei cannot produce ribosomes. Some cells, such as those of the alga *Acetabularia,* can be enucleated easily. In *Acetabularia* the nucleus is conveniently located in a rootlet or rhizoid at one end of the large cell. This rhizoid can be amputated without injury to the rest of the cell. The enucleated cell goes on making enzyme molecules, since it possesses ribosomes in plenty; however, it cannot produce any more ribosomes. But isolated nuclei possess the power of making new ribosomes, when supplied with the building blocks for making ribonucleic acid.

Considerations of a still more general nature also indicate that the ribosomes must at least obtain their information from the nucleus. We have known for many years that the chromosomes, each made up of many genes, are to be found in the nucleus. We know, too, that the genes—the genetic material of the nucleus—contain the information that determines that the cell is a cell of a particular species of organism, instead of some other kind. The genes contain information specifying flower color, leaf shape, and so on. George W. Beadle and his colleagues at Stanford have clearly shown that the function of the genes is to determine what kind of enzyme molecules are made by the plant cell. In fact, there is an expression today, "one gene-one enzyme," which means that one gene contains the information required to determine that that cell makes one kind of enzyme. A second gene contains information required to determine that the cell makes a second kind of enzyme, and so on. We know this from the fact that a slight alteration or mutation in a gene causes a cell to make an altered or mutated enzyme molecule. Since we are aware that enzyme molecules are made by ribosomes, it is therefore clear that the ribosomes must obtain their information content from the gene. How does this happen?

Before we can answer the question of how the genes make the ribosomal tapes, we must first know that the genes are made of special gene material. This gene material is another kind of nucleic acid—deoxyribonucleic acid (or DNA, as it is called). The genetic DNA is again a long-chain molecule made of four kinds of building blocks which can succeed one another down the chain in any one of many permutations and combinations. It is believed, although it has not yet been rigorously demonstrated, that the genetic information is contained in the sequence with which these building blocks of DNA succeed one another down the chain.

The four kinds of building blocks of DNA closely resemble the four kinds of building blocks of RNA, although with one specific and characteristic difference. The building blocks of DNA lack a specific kind of chemical group, a particular oxygen-containing group, which is found in the building blocks of RNA. This oxygen-containing group of RNA appears to be essential to the art of enzyme formation. As far as we know today, DNA does not possess the power to make enzyme molecules directly; this role is reserved for RNA. However, the absence of the oxygen-containing group from the building blocks of DNA confers upon DNA a new and

interesting property that RNA does not possess: DNA is able to reproduce itself. DNA can replicate, conserving intact the information-containing sequence of building blocks, the sequence in which the genetic information is written.

DNA is composed of chains of building blocks—adenine, thymine, guanine, and cytosine—which may be symbolized by A, T, G, and C. However, the DNA molecule actually is not a single chain, but rather a double one. In this double chain, there is a specific and characteristic relationship between the sequences of letters in the two strands. A basic selection rule is at work—perhaps the most basic rule of all biology—which says that wherever there is an A in chain number 1, there must be a T in chain number 2. Where there is a G in chain number 1, there must be a C in chain number 2. A pairs with T; G with C. By this pairing arrangement, the two chains of the double-stranded DNA molecule fasten together tightly and firmly to form a uniquely coiled and stable structure called the DNA double helix. We may visualize the replication of DNA in the following terms. We imagine that when the DNA molecule is to be replicated, it first becomes unraveled at one end into two single strands. A supply of the building blocks, A, T, G, and C, is available in the nucleus for making more DNA, and from this pool of building blocks the two unraveled single strands of the DNA molecule now proceed to assemble upon themselves new mate chains. In this assemblage, A pairs with T, and G pairs with C. The unraveling of the original double chain and the assemblage of new mate chains proceed down the length of the original DNA molecule until the process is consummated and there are two new double chains, each identical in structure with the original parent. The property of replication with conservation of information is a unique and wonderful property of DNA—a property that makes DNA the basic substance of life. DNA contains information, and it can replicate this information. DNA may be thought of as a do-it-yourself book containing all the information on how to make a cell and, in addition, a do-it-yourself book that can multiply itself.

This view of how the DNA replicates itself has been established by many kinds of experiments. Among the most direct, and one not too difficult to do, is the experiment whereby DNA may be caused to replicate in a test tube. If we put some DNA into a test tube together with some of the building blocks, A, T, G, and C, wait a little, and after a short time look to see how much DNA is in the