Biotechnology: Implications for Horticulture and Society

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"Biotechnology" is a word that probably has no equal in meaning so much or so little to so many. However, it does convey an exciting concept in that we have new tools and technologies enabling us to modify living organisms with a precision not formerly possible, and to combine traits from organisms that are unrelated or incompatible.

Biotechnology, broadly defined, includes any technique that uses living organisms or parts of organisms to make or modify products, to improve plants or animals, or to develop microorganisms for specific uses (1). Using this definition, biotechnology can be said to have originated at least 10,000 years ago when the transition was made from a food-gathering society to one which cultivated plants and domesticated animals. The early farmers selected crops and animals for desirable traits which improved productivity or adaptability to a given environment. Thus, the earliest farmers were taking advantage of genetic variability inherent in plants and animals. They also developed the biotechnology of fermentation to produce wines, beer, and sauerkraut and the use of yeast to make bread. More recently, microorganisms have been used to produce antibiotics to control disease.

So what, then, is new and the basis for all the excitement? What is new are 2 separate developments, each having its genesis in the 1940s. I will trace each development briefly and show how they have now come together, bringing us to one of the most exciting times in the history of plant science research.

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Cell and tissue culture

The first development is the discovery of cytokinins and the hormonal control of shoot and root regeneration from tobacco callus by Skoog and his coworkers in 1948. A practical use of tissue culture was the production of virus-free plants from meristems. Using this technique, Morel in 1964 found that shoot tips from cymbidium orchids proliferated into masses of protocorms which could be divided and recultured to produce new plants. In the same year, F.C. Steward at Cornell Univ. reported that carrot callus produced in tissue culture could be separated into single cells, and that a single cell could be regenerated into a whole plant. Thus, the concept of totipotency was introduced—that is, a single cell contains all of the genetic information that is needed to form a total plant. J.P. Nitsch reported in 1969 that he could regenerate a plant from a pollen grain and thus produce a haploid plant. Other scientists found they could culture protoplasts following the enzymatic digestion of the cell wall. As scientists began to master the art and science of plant tissue culture, increasing the number of plants that could be regenerated from single cells, they found that there were some unexpected variations in the progeny or what is called "somaclonal variation". It is now recognized that the rate of mutation increases and can be expressed more easily when culturing large populations of plant cells. The selection pressure can be increased by placing the cell population under conditions of stress, such as the presence of high salts, a toxin from a disease-producing organism, or low temperature. It is possible, therefore, using the culture of cells or cell protoplasts to speed up the process of finding and isolating genetic variability. Plants can be selected which are more adaptable or resistant to disease in a shorter time and in less space than conventional approaches require.

Cell and tissue culture also made it possible to begin asking some of the fundamental questions about plant differentiation. What are the mechanisms regulating the expression of genetic information contained in the cell nucleus? What causes a group of cells in an undifferentiated mass of callus to become organized into a shoot or root? What causes a single carrot cell to develop into an embryoid and eventually into a plant? There is still much to be learned about cell and tissue culture. I referred to tissue culture as an art and a science because, currently, both are required for success. The number of species that can be regenerated from single cells is still limited, and the traits selected from somaclonal variation are not always stable. But the fact that there are some 23 papers at this joint ASHS–CSHS meeting devoted to cell and tissue culture indicates the high level of interest. It should be recognized that horticulturists have made major contributions in bringing us to our present state of knowledge in the field of cell and tissue culture.

Recombinant DNA technology

Now let us turn to another arena of science which was evolving in the same time period, often in conjunction with health-related research, using microorganisms as the research tool. This is the area of recombinant DNA technology. Starting in the 1940s, Oswald and others presented evidence that genes were made of deoxyribonucleic acid (DNA), a molecule consisting of sugar phosphate and 4 nucleotide bases: adenine (A), guanine (G), thymine (T), and cytosine (C).

In 1953, Watson and Crick described the 3-dimensional structure of DNA as a 2-stranded molecule coiled in a double helix (Fig.1). The nucleotide bases stick out from the string of sugar and phosphate which makes up the backbone of the molecule. The 2 strands are held together by weak bonds between the bases. Each strand is complementary to the other with A binding with T and G with C. The order in which the bases are

[1]Presented at the joint 81st Annual Meeting of the American Society for Horticultural Science and 29th Annual Meeting of the Canadian Society for Horticultural Science (CSHS), Vancouver, B.C., on 6 Aug. 1984.

[2]Hess (MS, PhD, Cornell Univ.) held academic appointments at Purdue Univ., Rutgers Univ., and Cook College before accepting his current post in 1975. He has received numerous awards for his work in the physiology of plant growth regulators, particularly those affecting root formation. He has served on state, national, and international advisory boards and commissions, including President of ASHS (1973). In 1982, he received a Presidential appointment to a 6-year term on the National Science Board, of which he was elected Vice Chairman. In 1984, he was appointed by the Governor to the California State Board of Food and Agriculture for a 4-year term.

arranged on the backbone is the "code". TAC, for example, is the code for the amino acid methionine; TAT is the code for isoleucine. Sequences of amino acids form proteins which serve as structures such as membranes or as enzymes which catalyze metabolic reactions within the cell.

Genetic information contained in the nucleus is expressed in the cell by the processes called transcription and translation (Fig. 2). A portion of the double-stranded helix unwinds and one strand serves as a template for the formation of a complementary strand of messenger ribonucleic acid (RNA). The process is similar to DNA replication with the exception that RNA has uracil as a base in place of thymine. Once transcribed, the messenger RNA moves from the nucleus to the cytoplasm where it attaches to ribosomes, and proteins are synthesized according to the amino acid sequence encoded on the messenger RNA strand.

How then does an individual select a single gene from among the many thousands or more genes occurring along the strands of DNA that make up a chromosome? In the 1970s, scientists found (in bacteria) restriction enzymes which cut DNA strands into pieces, thereby eliminating foreign DNA. The restriction enzymes each have a unique specificity for nucleotide sequences. The enzyme will cut the DNA strand only when it locates a specific sequence.

For example, the Eco restriction enzyme recognizes the sequence $\frac{\text{CTTAAG}}{\text{GAATTC}}$ and cleaves each strand between G and A (Fig. 3). The single strand ends join readily with other DNA fragments cleaved by the same enzyme; they are called "sticky ends". By selecting the proper restriction enzyme, it is possible to remove a selected gene from the donor DNA molecule. It is also possible to use the restriction enzyme to open a plasmid (circular strands of DNA) and then insert into this opening the gene removed from the donor DNA. If the plasmid is inserted into a bacterium and the gene is expressed, large amounts of the specific protein will be produced following multiplication of the bacteria (Fig. 4).

Merging the 2 technologies

In addition to inserting a gene into a bacterial plasmid for "cloning", it may be inserted into a higher plant. Three methods are currently available to insert the new genetic information into a plant as shown in Fig. 5: 1)direct insertion into a protoplast; 2)use of a virus; and 3)the use of the crown gall bacterium, *Agrobacterium tumefaciens*, as a vector. After the gene is inserted into a cell, tests have to be conducted to see if it is expressed. The foreign gene must be attached to a "promoter region" along the plasmid, that is, a part of the DNA controlling adjacent genes. The next step is to regenerate a plant from the transformed cell and then determine if the foreign gene functions in the intact plant. It is at this point that the 2 areas of science come together—the merger of recombinant DNA technology and plant cell and tissue culture. The final test is to see if the foreign gene is passed on to the progeny following sexual reproduction.

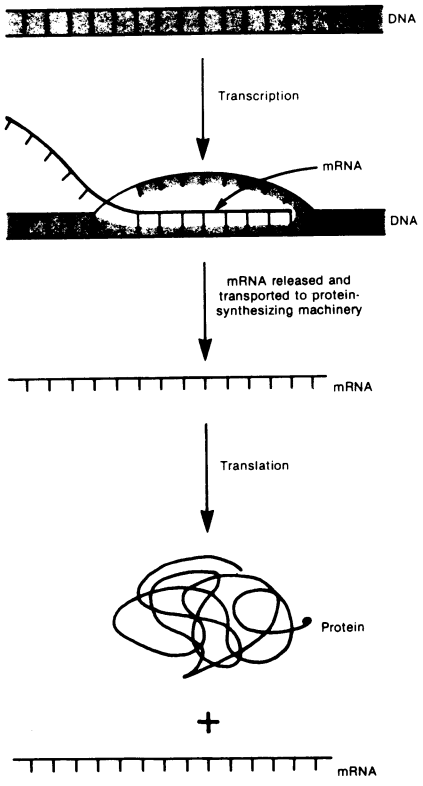

Fig. 2. Mechanism of gene expression (1). Source: Office of Technology Assessment.

Let me return for a moment to a little more detail about *Agrobacterium tumefaciens*. This bacterium is a very interesting, naturally occurring genetic engineer. The crown gall bacteria invade the tissue of susceptible plants through wounds. Once in a plant cell, part of the plasmid, known as the Ti (for tumor-inducing) plasmid, is inserted into the plant cell's genome where it is replicated and expressed along with the plant's DNA. That is why, once infection occurs, the tumor-forming characteristic persists even in the absence of the *Agrobacterium tumefaciens*. In addition to introducing genes which stimulate tumor formation, a gene is introduced which causes the synthesis of unusual amino acids (opines) which only the *Agrobacterium tumefaciens* can use as a food source. Therefore, we have a naturally occurring form of genetic engineering in which a bacterium is able to modify its host and cause it to produce a substance only it can use as a food source.

It is possible to inactivate the tumor-forming characteristic of the plasmid and insert other genes which carry desired characteristics. In Jan. 1983, a research team at Monsanto inserted a bacterial gene for antibiotic resistance into the Ti plasmid, which was then used to transform petunia cells in culture. Plants regenerated from cell culture carried the antibiotic resistance and, most importantly, the antibiotic trait was carried through sexual reproduction into the subsequent generations. The experiment passed the required tests I mentioned earlier.

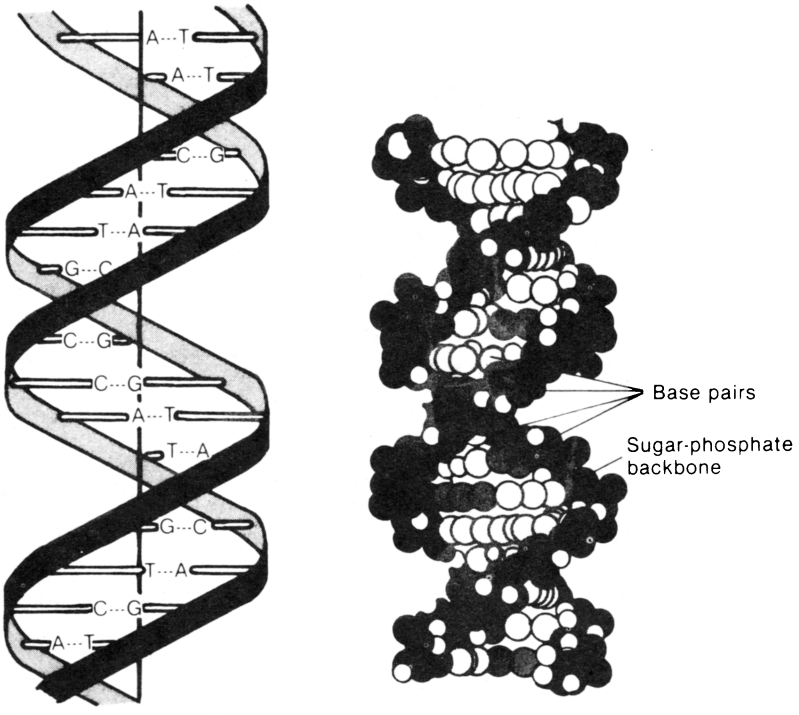

Fig. 1. The structure of DNA (1): A schematic diagram of the DNA double helix (left); and a 3-dimensional representation of the DNA double helix (right). Source: Office of Technology Assessment.

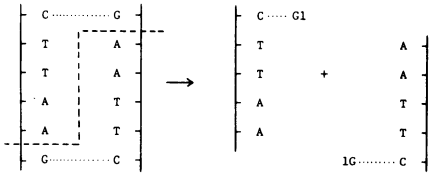

Fig. 3. Restriction enzyme cleaving DNA.

Therefore, we have in theory (and, in some cases, in practice) the ability to take a specific gene from a bacterium or other organism, insert it into a plant cell genome, and have it expressed in the regenerated plantlet and in subsequent generations. The technology is available, then, to isolate single gene traits (such as dwarfness, disease resistance, and herbicide resistance) from one organism and to insert them into another organism which lacks these traits. In the future, it may be possible also to insert multigenic traits, such as biological nitrogen fixation, productivity, and resistance to stress.

Identifying important genes

The great challenge facing horticulturists and other plant scientists today is the identification of what gene or genes are important. Robert Goldberg of UCLA has stated that in a tobacco plant about 100,000 of the total 2 million genes are active at a specific time in the life cycle of the plant; that is, about 5% of the DNA contained in the nucleus is being transcribed into RNA and translated into protein. Some genes are active throughout the entire plant. Goldberg calls them "housekeeping genes". Other genes are expressed only in one organ. The petals and leaves each contain about 7000 specific genes. The ovaries and anthers contain about 10,000 specific genes (Fig. 6). A key question is what activates those 7000 or 10,000 genes so they are expressed only in the petals and leaves or in the ovaries and anthers, respectively. Cell culture can provide an important tool in trying to identify useful traits, such as salt tolerance or low-temperature resistance. Once a useful trait is found, 2 approaches may be used to find the genetic basis of that trait. One can try to find differences in the plant genome between resistant and nonresistant plants or, alternatively, the biochemical and physiological mode of action of the resistance can be determined to provide clues as to what gene or genes may be involved.

Biotechnology is also providing new opportunities to find answers for some of the great unsolved problems in biology and the plant sciences. As mentioned earlier, the question of how differentiation is regulated still needs to be answered. *Agrobacterium tumefaciens* may provide some clues. In experiments using the Ti plasmid, selected removal of sections of the DNA resulted in callus cultures which exclusively produced shoots or exclusively produced roots. The new techniques of selective gene removal or insertion, combined with cell and tissue culture and the classical techniques of biochemistry and plant physiology, should help provide answers to the elusive questions of differentiation. These examples indicate that there has never been a greater opportunity or need for the establishment of multidisciplinary research teams in the plant sciences or for the blending of basic and applied approaches to a research problem.

Examples of using the new biotechnology

Several plants in the legume family form a symbiotic association with the soil bacterium, *Rhizobium*. The *Rhizobium* stimulates the formation of nodules in the root system providing a home for the bacteria. The plant supplies the bacteria with sugars from the photosynthetic activity. In turn, the *Rhizobium* bacteria take nitrogen from the air and fix it into ammonia which is released to the plant as a nitrogen source. It has been possible to identify the genes responsible for nitrogen fixation and to transfer them to *E. coli*, which in turn become nitrogen-fixing. The ultimate goal is to transfer the nitrogen-fixing ability to higher plants and to eliminate the need for a symbiotic relationship. The genes for nitrogen fixation and nodule formation have been isolated from *Rhizobium* and transferred to *Agrobacterium tumefaciens*. The *Agrobacterium* was used as a vector to insert the genes into an alfalfa plant. The genes regulating nodule formation were expressed, but unfortunately the genes for nitrogen fixation were not. If the genes are expressed, the productivity of the crop may be decreased because a portion of the plant's energy will be diverted to nitrogen fixation which, even in a biological system, requires a high level of energy. There are, however, intermediate steps which can improve the efficiency of the process. For example, about two-thirds of the *Rhizobium* bacteria in the United States are inefficient; in the process of biological nitrogen fixation, they release hydrogen gas. One-third of *Rhizobium* bacteria are more efficient because they have a gene, called the "hup gene", which causes the hydrogen to be recycled in the nitrogen-fixation process. It is now possible to transfer the hup gene into the inefficient native strains of *Rhizobium* bacteria, and then inoculate legumes with the modified bacteria. Since only one gene has been changed, the chances of the modified bacteria surviving under field conditions are good. Recently, the nodule gene has been transferred to *E. coli* which now can stimulate nodule formation. This is an example of the use of genetic engineering to gain new knowledge about host–microorganism relations.

Practical benefits can also be realized by the removal of the gene. Lindow at the Univ. of California is working with *Psuedomonas syringae*, a bacterium which lives in the epidermis of many plant species including beans and potatoes. The naturally occurring *Psuedomonas syringae* releases a substance which serves as the nucleus for icecrystal formation as the temperature drops to freezing. The ice crystals pierce the epidermal cells and cause injury. It was found that a gene could be removed and the modified bacteria no longer caused the ice nucleation. Greenhouse tests indicate that if the wild strain is replaced by the genetically modified bacteria, the plants will tolerate exposure to lower temperatures. The field testing of the modified bacteria has become the center of a legal action brought by Jeremy Rifkin, representing the Foundation of Economic Trends. Judge John Sirica

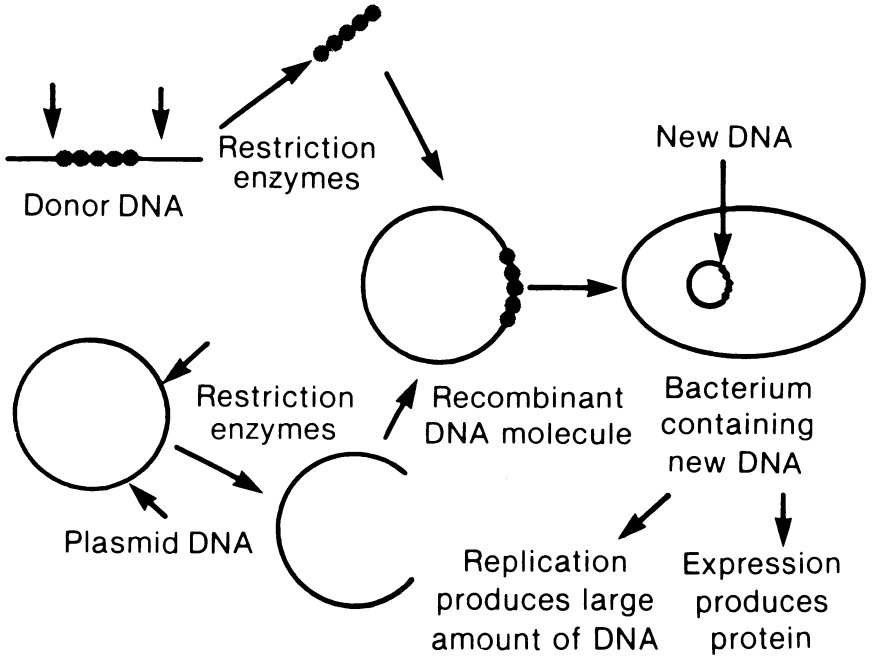

Fig. 4. Recombinant DNA: the technique of recombining genes from one species with those of another (1). Source: Office of Technology Assessment.

has issued a restraining order against release of the organism in the environment while he studies whether due process and proper environmental impact analysis was conducted by the National Institutes of Health's Recombinant DNA Advisory Committee.

Another example is a search for herbicide resistance. This work is being pursued actively by several laboratories, both public and private, including Calgene in Davis, Calif. The advantages of the broad spectrum herbicide, Roundup, are that it kills most if not all plants and is considered to be environmentally safe because it is inactivated quickly in the soil. By growing *Salmonella* bacteria in solutions of Roundup, however, it has been possible to obtain a strain of *Salmonella* which is tolerant to the chemical. It was found that the resistant bacteria had a single gene change modifying one amino acid in a protein which was capable of inactivating Roundup. The structure of the gene has been characterized and the goal is to transfer that gene into crop plants to make them resistant to Roundup. Then it would be possible to spray Roundup on the crop, eliminating the weeds with an environmentally safe compound, and leave the crop plants undamaged. At this point, although it is possible to insert the gene into plant cells, gene expression has not been achieved.

Problems and challenges

These examples give a sense of the promise and also the problems of the new biotechnology. The capability to introduce traits of biological nitrogen fixation, low-temperature resistance, or resistance to a herbicide has tremendouse promise. The problems and challenges are: 1) the identification of genes which determine the characteristics in which we are interested; 2) the ability to isolate those genes from among the 2 million or more other genes that exist in plant cells; 3) to find a vector to transfer the genetic information into a plant; and 4) to have the gene expressed and passed on through sexual reproduction into subsequent generations. These all remain formidable challenges. The challenge is not only in molecular genetics, but in conventional plant physiology, biochemistry, and plant breeding as well. The new biotechnology does not replace existing science, but adds a new tool for all of us to use.

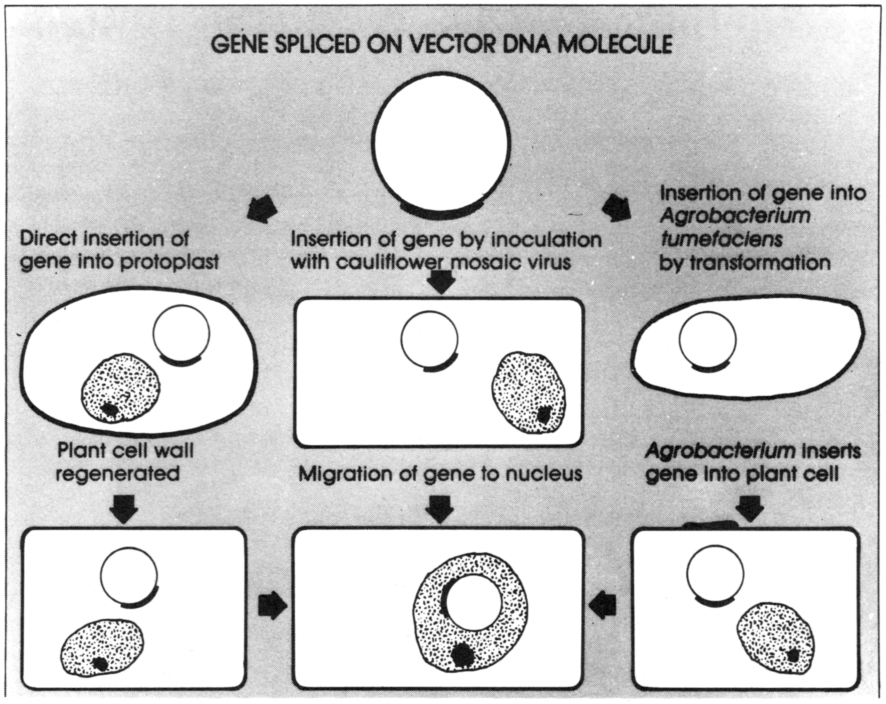

Fig. 5. Three methods of inserting a gene into a plant cell.

The new biotechnology does add an additional challenge to which we as academic scientists have not been exposed in the past, namely a much greater public awareness and concern for the research we are conducting, and the desire of some groups to play a role in determining what research will or will not be conducted. The ability to isolate and modify genetic information introduces many ethical, moral, and legal concerns. Individually as scientists, and collectively as a Society, we have a responsibility to ensure that all precautions are taken in conducting research so that the modifications we make do not create more problems than they solve.

Our other responsibility lies in our traditional role as educators. We must provide our students and the public with a better understanding of genetic engineering and its role in the plant sciences. The fact that we have been in the business of genetic engineering takes place in nature, and that we are sensitive to potential environmental and ecological impacts should help minimize the fears. Lack of information creates an environment in which fear can grow to unrealistic proportions. The growing interest in general education in our universities provides an opportunity and a responsibility for horticulturists and agriculturists to develop courses which will provide scientific and agricultural literacy for graduates in the social sciences and humanities. I think the excitement of the new technology and discussions of the moral, ethical, and legal issues should be very attractive to a broad range of students.

Similarly, we should take opportunities to discuss the subject with public groups. And, as a Society, we should be conscious of, and possibly become involved in, legal actions such as Rifkin's suit. At this point, the arguments are focused upon whether a proper environmental impact analysis was conducted, and whether ecologists reviewed the application to release the genetically modified organisms. Many supporters of this particular case, however, are opposed fundamentally to the new biotechnology. Unless we take an active role in educating our students and the public, and participating in a vigorous defense against the actions of individuals opposed to genetic engineering research, all the promise that the new biotechnology holds will not be realized.

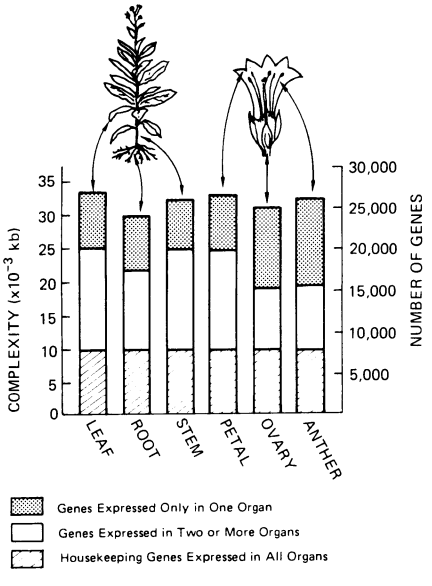

Fig. 6. Gene expression in plants. Source: Robert Goldberg, Dept. of Biology, Univ. of California, Los Angeles.

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