Progress is being made along a broad front in the engineering of rice and other important food crops. More is needed if insight is to be put to use.

Within the past two years, at least four research teams—from the University of Nottingham in England, the University of Tsukuba in Japan, Plant Technology (a Tokyo company associated with Mitsubishi Corporation), and Cornell University—have inserted novel genes into rice, the world’s most important cereal crop. These transformed rice cells were then regenerated, via cell-culture techniques, into whole plants. Because many earlier successes at plant transformation and regeneration involved experimental species of less commercial value than rice, the news that rice had been genetically transformed was received with enthusiasm.

Unfortunately, because the researchers do not know how, why, or where the transferred genes were incorporated into the rice genome, or what the regulatory controls were that allowed a single transformed cell to regenerate into a whole plant, they are unable to reliably repeat their achievements with other crops. Furthermore, the genes introduced into rice were marker genes, of no useful value to the plant.
The early days of plant molecular biology focused on the discovery of simple but powerful tools that allow the movement of genes from one species to another, thus breaking the species barrier that frustrated generations of classical plant breeders. (See “Toward a Biotechnological Revolution” by Anne Simon Moffat, Mosaic Volume 17 Number 2 Summer 1986.) The first genes transferred into plants were single genes that served as markers, which proved that the plant had, indeed, received foreign genetic material and had become transgenic. Such genes coded for discrete functions, such as resistance to the antibiotic kanamycin or to the anticancer drug methotrexate. Soon after, single genes were moved that coded for practical traits, such as the quality of their seed proteins or resistance to herbicides. (See “A practical science” accompanying this article.)

Now the discipline is maturing. Researchers are defining gene families, including the needed regulatory genes that encode complex plant functions, such as response to light, nitrogen fixation, and hormone production, and they are upgrading their tools to allow the transfer of such gene families. They also hope to expand their repertoire of tools so that a wide range of plants can be transformed genetically. To date, only two dozen plant species have been successfully transformed but only a few—rice, canola, cotton, soybean, and potato, for example—have significant commercial value. Petunia, tobacco, and Arabidopsis thaliana are genetically manipulated routinely, but they are of interest principally as model plants. (See “A model plant...” accompanying this article.)

Researchers also hope to systematize the cell-culture procedures needed to grow transformed cells into whole plants. An extraordinary trait that distinguishes plant from animal cells is their ability to regenerate into fully functional whole organisms from single cells. A genetically transformed cell, if placed in an appropriate brew of nutrients, minerals, and hormones, will form roots and shoots and grow. What has eluded researchers, however, is an understanding of what makes a cell-culture system work. Successful cell culture relies more on empirical trials than on firm scientific principles.

The potential rewards of such research are great. Fast and easy means for transforming and regenerating plants will allow the commercial production of new plant varieties that produce, for example, high-value oils and proteins for pharmaceuticals or microelectronics. (See “Circuits and Molecules a Molecule Wide” and “Devices that Assemble Themselves,” both by Mort LaBrecque, Mosaic Volume 20 Number 1 Spring 1989.) Most important, the design and manipulation of transgenic plants allow researchers to gain a better understanding of how plants grow and develop. Genes from distantly related plant species, bacteria, and mammals have been placed in plants, enabling researchers to selectively pick apart the plant genome to reveal the details of gene expression that govern plant growth and development. Once these complex steps are sorted out—a non-trivial task, indeed—plant biologists hope to redesign rice, or any other plant, with a predictability, reliability, and speed unknown to classical plant breeders.

Enthusiasm is widespread. (See “Breaking the doldrums” accompanying this article.) “The current excitement about plants as experimental organisms derives from the perception that plants represent a new biological frontier ready for experimentation,” says molecular biologist Robert Goldberg of the University of California at Los Angeles. “There are so many big plums to pick,” says R. Scott Poethig, a plant molecular biologist at the University of Pennsylvania. “There is no shortage of interesting problems. That is why so many scientists are switching from animal studies, where the field is getting crowded, to plant biology.”

Retooling rice

Scientists used four different techniques to engineer rice, each entailing some physical manipulation of the plant cell. The most novel of these employs a gene gun developed by John Sanford of Cornell’s New York State Agricultural Experiment Station; Ed Wolf, a Cornell engineer specializing in microminaturization; Nelson Allen, model shop supervisor, and Ted Klein, formerly of Cornell and now of the USDA’s Plant Gene Expression Center in Albany, Cal-
The Cornell gene gun propels DNA-coated tungsten microprojectiles with a .22-caliber blank shell through a partial vacuum into plant tissue.

Biologists. The Cornell gene gun propels DNA-coated tungsten microprojectiles with a .22-caliber blank shell through a partial vacuum into plant tissue.

California. The gun propels tiny projectiles of tungsten coated with DNA into plant cells. It first proved itself in 1988 by inserting genes that restored the energy-producing ability of mutant animal and plant cells that lacked this function. In one set of experiments, DNA-coated microprojectiles shot into deficient chloroplasts of green algae restored photosynthesis. In a second, the gene gun was used to restore respiratory capacity in the mitochondria of baker's yeast. This method for inserting genes has become known as biolistics, from biological and ballistic.

Sanford says a general advantage of the gene gun is that it works on any type of plant and can be used to bombard millions of cells at a time. It does not, however, allow scientists to target precisely. Current models are molecular shotguns, not rifles. Adding to the problem is the unstable nature of transformed cells. In spring 1989 Cornell sold the rights to the gun to DuPont.

The popularity of the gene-gun concept has encouraged others to work in the area. For example, Winston Brill, formerly of Agracetus in Middletown, Wisconsin, has a variant of the gun, which he describes as a "finely tunable, electrical-discharge particle accelerator" that blasts DNA-coated gold particles into plant cells. Soybean seed have been transformed with the Agracetus model. Laurens Mets of the University of Chicago has another gene-insertion device, which should be available by late 1989 or early 1990. His "aerosol beam injector" shoots DNA in a solution of either 10 percent polyethylene glycol (PEG)—a compound that eases the passage of DNA into cells—or 95 percent ethanol. Unlike the other guns mentioned, the injector gives a continuous beam, not a blast, and does not use metallic microprojectiles. "It is a very fast cloud," says Mets.

The most controversial method used to transform rice is the pollen-tube-pathway technique, which was used by Ray Wu of Cornell University. With this technique, DNA is flooded into the pollen tube, a natural conduit between the outside world and the developing embryo in the plant. Each plant is transformed individually, by carefully pipetting a few drops of DNA onto a flower's stigma, the female organ that receives pollen. This technique has the advantage of directly transforming seeds, not single plant cells. Such seeds can be grown into whole plants with less fuss and bother than is needed to coax a transformed cell to grow into a whole plant via cell-culture techniques.

The pollentate pathway technique, which was originally developed by Zhou Guang-Yu of the Shanghai Institute of Biochemistry, Academia Sinica, to transform cotton, lacks acceptance. Although some researchers believe the transformations achieved via the pollen-tube method are artifacts, this may be the easiest method of transforming plants, if the technique is verified. The only obvious drawback is that it is time-consuming.

The two other physical techniques used to transform rice involve the application of methods previously established to genetically engineer bacterial and animal cells. The first technique uses polyethylene glycol; the second is electroporation, in which a pulse of electricity punctures self-repairing holes in protoplasts, plant cells stripped of their cell walls.

Although each of the four techniques used to transform rice was developed independently, they are now being used in various combinations with plants to improve the efficiency of transformation.

Biological vectors

Interest in developing these tools for inserting genes into plant cells was spurred after it was realized, about four or five years ago, that the very first tool for transforming plants—a biological tool—had a major flaw: Although it could routinely transform broadleaf dicots, it was unsuccessful with grass-like monocots, which include all the valuable cereals. That tool is Agrobacterium tumefaciens, a soil-borne bacterial pathogen. Because of its ability to cause plant tumors known as crown galls, it became a focus of early cancer research during the decades of the 1940s and 1950s.

Agrobacterium was recognized as an extremely invasive organism; it could invade a wound in the cell wall around a plant cell with ease and insert its DNA into the plant genome, where it was expressed. Thus, to modern plant biol-
Within the last few years, interest has wanted genes. The first proof that such genes that cause plant cancers. During the early 1980s, however, researchers from Monsanto, CIBA-Geigy, the University of Washington, the Max Planck Institute in Cologne, West Germany, and the State University in Ghent, Belgium, devised a technique to deal with this problem. They mapped the plant oncogenes to a tumor-inducing (Ti) plasmid within the bacterium and then stripped Agrobacterium of these unwanted genes. The first proof that such a disarmed plasmid could transform plants was announced in 1983 by a team of Monsanto biologists—Robert B. Horsch, Stephen G. Rogers, and Robert T. Fraley—and also by the teams of Jeff Schell of the Max Planck Institute and Mark Van Montagu of the State University in Ghent.

To this day, however, it remains unknown why Agrobacterium selectively invades dicots. A few laboratories in the United States, Japan, and Europe are probing the elements that facilitate interaction between Agrobacterium and plant cells and those that block it. Within the last few years, interest has focused on the role of diffusible chemicals—present in dicots but perhaps absent in monocots—that may induce the transfer of genes.

Another early effort to design biological tools for transforming plants had mixed results. Attempts to manipulate whole plant viruses which, as pathogens, have excellent means for invading cells, have been more challenging than anticipated. However, the use of molecular bits of the viral DNA to regulate the expression of other introduced genes proved hugely successful.

Stephen Howell of the Boyce Thompson Institute for Plant Research in Ithaca, New York, who was the first to introduce recombinant DNA into plants in the form of the cauliflower mosaic virus (caMV) genome, says that “the use of caMV as a biological vector has been difficult because it is small and can carry only so much extra genetic baggage.” But, he adds, part of the virus known as the 35S RNA promoter, which activates genetic expression and regulates the time and locus of activation, is proving very valuable.

Recent work from the laboratories of Howell and Nam Hai Chua of Rockefeller University in New York City suggests that information gleaned from the 35S promoter offers important details about gene regulation. For example, using a series of deletion experiments, in which the promoter was chopped up and different fragments were hinged to a marker gene and inserted into transgenic plants, Chua’s lab distinguished the more important bits of the promoter from the less important ones. A change of just four base pairs was found to be enough to alter gene expression.

Moreover, the Chua team identified trans factors, which are cellular proteins that bind the genome and affect its transcription, i.e., the synthesis of RNA from the DNA template. “Our expectation is that by overexpression of the trans-acting factors, which bind to these key sequences, we can selectively turn on and off the expression of the target gene,” says Chua. A major goal is to probe the physical interactions between the protein trans factors and genetic material. This may yield information on general mechanisms of how genes are “bound” and controlled by cellular proteins. The Chua team has also isolated the gene that codes for one of the trans factors. “The promoter has proven to be a gold mine of information,” says Rockefeller’s Chua, “and its break-up value is greater than the sum of its parts.”

Other viruses are still candidates, albeit longshots, as biological vectors for transforming plants. Paul Ahlquist of the University of Wisconsin used the bromo mosaic virus as an expression vector in cultured barley cells. That is, the novel genes were inserted and expressed in the plant cells, but not integrated into the genome, so they cannot be passed to progeny.

**Homologous recombination**

Although a variety of biological and physical tools are available for inserting novel genes into plants, the techniques do not allow genes to be targeted to specific sites in the plant genome. Ideally, what is needed is homologous recombination, that is, the ability to target an incoming piece of DNA to a specific gene. Techniques have been developed for homologous recombination in yeast first and now in mice. (See “DNA on Target” by Ben Patrusky elsewhere in this Mosaic.)
But such exquisite systems have not yet been worked out for plants. Plant biologists must, therefore, contend with the fact that the available tools insert genes into the genome at random, making manipulation of plants more difficult. Still, this handicap has been sidestepped thanks to the design of other ingenious means of control.

Anti-sense

One system for regulating gene activity that has attracted great interest in recent years is a technology known as anti-sense. In short, anti-sense compounds block the activity of unwanted genes. The technique got its name because the first anti-sense compounds were bits of "backwards" RNA that were designed to be complementary to select cytoplasmic RNA; in essence, they work by binding and blocking its ability to function. Preventing RNA from carrying out its routine work is tantamount to turning off the gene that encoded it.

The technology got its start about ten years ago when P. C. Zamecnik of the Worcester Foundation for Experimental Biology in Shrewsbury, Massachusetts, suggested the use of synthetic compounds, known as oligonucleotides, to disrupt single-stranded RNA functions. Soon after, it was shown that anti-sense mechanisms could control gene expression in procaryotes, which are primitive organisms, that do not have organized nuclei.

Critical advances were made in 1984 when Harold Weintraub of Seattle's Fred Hutchinson Cancer Research Center and Douglas Melton of Harvard University showed that anti-sense RNAs could be used to block gene function in the more advanced eucaryotes—cells with an organized nucleus. To date, most anti-sense studies have been in medicine, but recently the technique was adapted for plants. In 1986, Ron Davis of Stanford University and Joseph Ecker, now of the University of Pennsylvania, showed that anti-sense RNA has the capacity to block the expression of plant genes.

In 1988, Donald Grierson of the University of Nottingham School of Agriculture in Loughborough, England, published a paper on the use of anti-sense RNA in tomatoes to decrease the production of the enzyme polygalacturonase (PG), which accelerates the breakdown of cell walls and causes mushiness. This was the first practical application of anti-sense RNA in plants.

"We are delighted to have used anti-sense techniques to selectively tamper with one gene, among 80,000, with no nasty side effects," says Grierson. Shortly afterward, Calgene, located in Davis, California, received the first U.S. patent covering the use of anti-sense technology in genetically engineered plants—also for work on a PG system in tomatoes. Calgene's research was aided by the Campbell Soup Company, which has an interest in cutting losses attributed to bruised tomatoes.

Lawrence Bogorod and Steven Rodermel of Harvard University are using anti-sense techniques to study the regulation of the plant enzyme ribulose biphosphate carboxylase—better known as rubisco, the carbon-dioxide-fixing enzyme. It catalyzes reactions in both photosynthesis and photorespiration and may be the most abundant protein on earth. Rubisco consists of small and large protein subunits. The small ones are encoded by a multigene family in the nucleus, and the large by copies of a single chloroplast gene.

Techniques for selective tampering with multigene families have not been developed. However, Bogorod used anti-sense to reduce expression of the
Transposons have been used experimentally as mutagens in drosophila since 1982 and, recently, Nina Fedoroff of the Carnegie Institution of Washington, developed systems for doing so in select plants. However, in order to get the system to work, the transposon must shift into a preferred spot. Since transposons can be irascible—there is no established means for getting a transposon to behave on cue—it may be necessary to look at hundreds or thousands of plants to find a transposon that has moved to the desired spot and exerted the desired control. Success with transposons often requires generous greenhouse space, perseverance, and serendipity.

Identifying genes worth moving is a challenge to the designers of transgenic plants. The first traits to be manipulated in plants, such as those that code for herbicide resistance, were those governed by single genes whose protein products were known. Now a major hurdle facing plant biologists is the need to identify genes that affect important phenotypic traits—such as height, taste, and drought tolerance—and that are governed by gene complexes whose protein products are not yet known.

One technique for identifying these genes is restriction fragment length polymorphism, or RFLP. This technique, which maps traits by comparing the genetic fragments produced by enzymatic digestion of genomes from different phenotypes, was proposed in 1980 by David Botstein, now of Genentech in San Francisco, Ray L. White of the University of Utah, and R. W. Davis of Stanford University. This is one of the techniques used for mapping the human genome.

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Another tool for affecting gene expression is transposons, the movable "jumping genes" first described by Barbara McClintock almost 50 years ago. Transposons have been used experimentally as mutagens in drosophila since 1982 and, recently, Nina Fedoroff of the Carnegie Institution of Washington, developed systems for doing so in select plants. However, in order to get the system to work, the transposon must shift into a preferred spot. Since transposons can be irascible—there is no established means for getting a transposon to behave on cue—it may be necessary to look at hundreds or thousands of plants to find a transposon that has moved to the desired spot and exerted the desired control. Success with transposons often requires generous greenhouse space, perseverance, and serendipity.

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### Breaking out of the doldrums

Optimism in modern plant biology is relatively new. The discipline had a heyday from the late 1920s to the early 1950s when the first plant hormones were discovered and isolated and their gross activity was described. Then the doldrums set in.

"From 1950 to 1975, there were not many individuals who made a living opening up plant cells and asking what is going on," wrote Leon Dure of the University of Georgia in *Opportunities for Phytochemistry in Plant Biotechnology* in 1988. "I imagine that in 1960 there were ten times the number of individuals studying metabolism in liver than in all plants combined. . . . Most textbooks treated plant cells as animal cells with chloroplasts encased in wood."

Things changed in the late 1970s when some companies, eager to capitalize on the new discipline of biotechnology, realized that profits might be made by applying newly developed molecular techniques to agriculture, thus creating a new discipline known as plant molecular biology. A few large firms such as Monsanto and DuPont invested heavily in the plant sciences, as did a few dozen start-up firms in Europe and the United States. "The scientists who left academe to staff these new laboratories are real heroes," says the University of Georgia's Dure. "Plant molecular biology would not have made it off the ground without them."

Early successes encouraged more support. Three private foundations—the Samuel Roberts Noble Foundation in Ardmore, Oklahoma, the McKnight Foundation in Minneapolis, Minnesota, and the Rockefeller Foundation in New York—made sizable commitments to the plant sciences. In addition, several federal agencies, including the National Science Foundation, the Department of Energy—which has a long-time interest in photosynthesis, the process used by plants to convert sunlight into chemical energy—and the U.S. Department of Agricul-
was among the first to use RFLPs with plants, providing the most detailed genetic map ever of the tomato. To date, this tool has also produced detailed maps of maize and *Arabidopsis*. (See "A model plant..." accompanying this article.) It has also produced the first ever genetic maps of pepper, soybean, potato, and lettuce. Rice is receiving detailed study in several laboratories. Unfortunately, the technique requires a considerable amount of repetitive work.

A different scheme for finding genes whose protein products are unknown has been proposed by Kenneth A. Feldmann of DuPont and Ralph S. Quatrano (who recently left DuPont for the University of North Carolina). The procedure involves the insertion into the *Arabidopsis* genome of a bit of marked DNA at every several thousand base pairs, thus creating a library of insertion mutants that could be used to identify unique traits and, subsequently, to isolate the DNA coding for the trait. To accomplish this, Quatrano says, a large effort is needed to generate about 70,000 plant transformants.

"It will take a large center, with the capacity to do 'brute force' genetics, to build this library of mutants and to screen it for specific traits," says Quatrano. "I hope an organization such as the USDA or a private company such as DuPont will take it on." The results, he says, would be a "valuable public resource for unique plant genes."

**Cell culture**

Although dramatic progress has been made in the development of techniques for identifying important plant genes and for inserting them into new species with a modicum of control, less progress has been made in cell culture, the process used to regenerate a transformed cell into a whole plant. A transformed cell—a technical triumph—has limited worth unless it can be coaxed into growing into a plant.

Theoretical underpinnings to cell culture are lacking. Some transformed cells, when placed in a cell culture medium, will grow. Many do not. "The bottleneck in plant molecular biology is the capacity to regenerate transformed cells into whole plants," says Ingo Potrykus, a leading plant scientist at the Swiss Federal Institute of Technology in Zurich.

To be sure, there have been notable achievements in the field of cell culture. Successful systems have been developed for culturing fertile plants of two important monocots—rice and corn. The latter success was announced in June 1989 by separate teams at DNA Plant Technology in Cinnaminson, New Jersey, and at CIBA-Geigy in Research Triangle Park, North Carolina. But Carol Rhodes of Sandoz Crop Protection in Palo Alto, California, who in 1988 was the first to design a successful cell-culture system for corn (although none of the plants were fertile), offers a caveat on this work. Writing in the June 1989 issue of *Bio/Technology*, she says, "What remains mysterious is how

exactly to induce the competent state (for regeneration) in maize cultures.... While the authors provide complete descriptions of the history of each donor culture, it is unknown what steps were critical in creating 'correct' physiology." In other words, cell culture still remains largely an empirical science.

Some plant biologists are attempting to "solve" the cell culture problem by eliminating the need for it. One such strategy is to directly transform embryos in seeds. These seeds grow by conventional means with no need for cell culture. Gene guns are being used to target novel genes into embryos or into the immature meristematic tissue that will become the female reproductive organ. A related strategy is to transform pollen. To this end, Potrykus is attempting to microinject pollen with novel genes, and Cornell's Ray Wu and Shanghai Institute of Chemistry's Zhou Guang-Yu are flooding pollen and pollen tubes with DNA. Both Wu and Zhou claim success with this method, Wu with rice and Zhou with cotton.

**Hormones...**

A few laboratories are attempting a more direct approach to the problem. A successful cell culture tricks a cell into accelerated development. By some unknown means, cell culture turns on the genes needed for growth and development. Plant hormones have a similar effect; they, too, regulate growth and development. So it seems that an understanding of hormone mechanisms may reveal the vital clues needed to crack the cell culture problem.

Although a detailed understanding of hormones remains a huge, unsolved puzzle, in the last few years researchers have used the tools of molecular biology to piece together a few key bits. Auxin, the cytokinins, and abscisic acid, or ABA, are three classes of plant hormones that are better understood as a result of such work.

Researcher Howell at Boyce Thompson Institute for Plant Research, for example, is probing the way the plant hormone cytokinin stimulates shoot growth in regenerating plants. "Inducing root growth is often easy in tissue culture," says Howell. "But cytokinin-induced shoot growth can be tougher, especially for some important crop plants." His lab showed that the addition of cytokinin to tobacco cells...
resulted in the rapid appearance of mRNAs that code for enzymes in the biosynthetic pathways of other hormones and growth factors. “We are seeking a network of hormones and a unifying mechanism that explains how they work together,” he says.

It has been known for some time, from the work of University of Georgia’s Leon Dure, that ABA can delay precocious germination in seeds. Recently, work in Quatrano and Dure’s laboratories showed that ABA may affect those genes that work in the developing embryo and in dealing with drought. That the same genes may affect both these functions is no huge surprise since the physiology of living, but dry seeds has parallels to that of water-stressed plants.

Quatrano, Dure, and others have also shown that ABA affects the accumulation of embryo-specific mRNAs. But they have chosen different, albeit complementary, approaches to probing the cascade of events between entry of ABA into a cell and continued embryogenesis and/or the mobilization of cell systems to endure water shortage.

Dure’s strategy is to learn everything possible about the proteins coded for by the mRNAs that appear in response to ABA. Most recently, Dure found a similarity in the amino acid sequences of such proteins in several species. They code for hydrophilic structures that “may represent domains functionally important in desiccation protection,” he says. But plenty of work remains.

“It is a backbreaking job just to get the milligrams of protein to do the chemistry,” Dure says, adding that the recruiting of talent to complete the job may be a problem. “Most graduate students want to manipulate genes,” says Dure. “They are terrified of proteins…. It does not hurt to point out that DNA does nothing. Proteins do all the work, and if we don’t understand how the proteins work, we really don’t understand the process.”

In contrast, Quatrano will study mutants of Arabidopsis that have an unusual response to ABA, such as an insensitivity to it, to learn how this hormone can trigger different responses in

A practical science

“The first genetically engineered plants were designed to improve crop protection,” says Barbara J. Mazur, manager of biotechnology research for DuPont. “Better resistance to herbicides, insects, and disease were the goals. The next phase of work will focus on improving crop development.”

That, she says, involves the redesign of plants to produce more high-value products, including medicinal proteins and industrial oils, and to better meet consumer tastes by modifying a food crop’s sweetness and spiciness, for example. The development of herbicide-resistant crops is the most advanced, says Mazur. The first of these crops was created by conventional plant breeding and cell-culture techniques (cell culture occasionally reveals desirable mutants) and should be commercially available in two to five years. “But many examples of transgenic herbicide-resistant crops have been reported and are in various stages of evaluation and development,” she says, including cotton, tomato, and canola.

Insect-resistant plants have been engineered by manipulating the workhorse Bacillus thuringiensis (Bt), a bacterium used for almost 30 years as a biocontrol. It produces a protein toxin that is lethal to insects. In the past, Bt was used as an insect control by spraying crystals of the Bt toxin onto crops. But now, thanks to genetic engineering, the gene for the Bt toxin can be inserted into plants, allowing the plants to produce the toxin endogenously. Several companies have genetically engineered a variety of crops such as tomato, cotton, and potato, to produce the Bt toxin. They include Monsanto, Plant Genetic Systems in Belgium, Rohm & Haas in Philadelphia, Crop Genetics International in Hanover, Maryland, and Ecogen in Langhorne, Pennsylvania.

In 1985, Roger Beachy of Washington University showed that plants could be made virus-resistant if they expressed a gene that codes for viral coat protein. Although elucidating the mechanism of this resistance remains of great interest, the commercial value of viral resistance may be limited since viruses mutate rapidly. However, the trait may be coupled with others and sold.

Although these recombinant plants have their market niches, none has the potential blockbuster status of either growth hormones or recombinant tissue plasminogen activator, or TPA, which breaks up blood clots and has a wide range of medical applications. The second generation of these recombinant plants, which should produce varieties with high-value carbohydrates, oils, and proteins, may yield as high a profit, however.

The Calgene Company in Davis, California is already manipulating the canola genome to produce high levels of erucic acid, an industrial oil used in the manufacture of rubber and plastics and as a cutting lubricant for steel and metal work.

The firm DNA Plant Technology in Cinnaminson, New Jersey, has isolated the genes for antifreeze peptides from arctic fish, and plans to put them into plants. Frost resistance appears to be a company specialty. It recently merged with Advanced Genetic Sciences, located in Oakland, California, best known for its recombinant pseudomonas bacteria Frostban, which prevents frost injury.

And, in what may be the most lucrative botanical genetic manipulation to date, Plant Genetic Systems in Ghent, Belgium is genetically engineering canola to produce high-value medicinals, including neuropeptides (e.g., the painkiller enkephalin), blood factors, and growth hormones.

Howard A. Schneiderman, vice president for research and development at Monsanto, is a firm believer in the potential of recombinant plants to produce renewable sources of food, fiber (for clothes and shelter), and pharmaceuticals.

“If you look back a thousand years from now, computers, microprocessors, lasers, and all that stuff is going to be in some Smithsonian basement,” he predicts. “But it is absolutely clear that human beings will be using genetic engineering to persuade crops and other organisms to produce the things we need. It will be our central way of making things.”

A.S.M.
different tissues. One hypothesis—"the branched hypothesis"—envisions the existence of a single receptor for the hormone, but different pathways of reactions leading from it into different tissues. Another—"the parallel hypothesis"—envisions multiple, tissue-specific receptors for the hormone, each linked to a different response pathway. With luck, the pathways will be short and amenable to a genetic manipulation. In either case, identifying the receptor(s) for ABA is a key goal of Quatrano's effort to define the mechanism.

... and helpers

One of the more provocative ideas about hormones is that carbohydrate fragments of the plant cell wall can act as hormones. Plant hormones, such as auxin and gibberellin, might work by activating an enzyme that releases specific carbohydrate messengers from the cell wall. Peter Albersheim and Alan G. Darvill, both of the University of Georgia, are the strongest advocates of this controversial idea. But, because the details of plant carbohydrate metabolism remain unknown, this idea will take time to verify.

Meanwhile, Albersheim reports progress in his work identifying the structures of oligosaccharides that trigger growth. He speculates that "there may be 100 or more growth regulators." His goal: to find the receptors for these compounds and the genes that code for these receptors.

Recent findings by other laboratories, however, have indicated a "hormonal influence" held by a probable component of the cell wall that is not a carbohydrate. For more than 30 years, a bitter controversy has raged over whether cytokinins alone could stimulate cell division. Andrew Binns of the University of Pennsylvania, Henry Wood of Rockefeller University, and David Lynn of the University of Chicago have shown that a group of natural products in plants, called dehydrodiconiferyl glucosides, might also mediate cell division. "There is a conceptual similarity between what Albersheim says and our work," says Lynn, "but the specifics are different."

Auxin's role

Within the past two years, a small group of researchers has clarified the role of auxin in the cell. This plant hormone was first isolated and chemically characterized in 1933 and, in the early 1950s, Folke Skoog's group at the University of Wisconsin showed that the concentrations and ratios of auxins to cytokinin could alter not only the growth pattern but also the relative levels of DNA and RNA in differentiated plant tissue. In 1954, Skoog made the then bold suggestion that plant hormones might work by affecting the metabolism of nucleic acids. Work followed showing that auxin enhanced RNA synthesis and that inhibitors of RNA and protein synthesis inhibited auxin-induced cell elongation.

In a paper presented in 1969, M. L. Evans and P. M. Ray, both of Stanford University, discounted, on the basis of theoretical considerations, the role of gene expression in auxin action. Their paper directed the focus of hormone research away from studies of the role
Arabidopsis, a model plant

The genomes of plants are huge—most of them ten times the size of those of many mammals and 100 to 10,000 times the size of bacterial genomes. Virginia Walbot of Stanford University and Sarah Hake of the USDA Plant Gene Expression Center in Albany, California have suggested that a corn plant is as genetically diverse as a human, despite much less obvious complexity.

Much of what might appear to be excess plant DNA may be needed to guide a plant's complex regulatory signals. Plants, which cannot physically escape from cold, drought, or predators, may use sophisticated genetic switches to trigger responses that allow them to cope with various stresses. Thus, while animals may respond to stress on a gross level by fleeing, fighting, or hibernating, there is growing belief that plants respond to stress in more sophisticated and subtle ways—at the molecular level.

Because plants have so large a load of DNA, they can be tough to work with. For this reason, plant biologists have sought a genetically leaner model plant. That plant is Arabidopsis thaliana. Its virtues include a smaller genome (five haploid chromosomes), little excess genetic baggage, fast growth, and a small mature-plant size, all of which allow it to be easily cultivated in greenhouses. Arabidopsis can be transformed by agrobacterium vectors and can be regenerated from protoplasts, plant cells stripped of their cell walls. Also, the small size of its seed—10,000 can be germinated in a single Petri dish—allows rapid and easy screening for new mutations, which offer a key to understanding normal plant growth and development.

If the ultimate goal of genetics is to understand what every gene does and how it is regulated, then it makes sense to study a higher organism with a relatively simple genome, such as Arabidopsis. To that end, an interagency effort, involving NSF and the USDA for starters, has been organized to establish a strategic plan for mapping and sequencing Arabidopsis. One research strategy being considered involves parceling out the sequencing chores by functional interest. For example, researchers with a special interest in flowering would map the relevant genes; those with an interest in photosynthesis would map a different set.

The first time estimate established by the planning group suggests a complete Arabidopsis map by the turn of the century. A.S.M.

of auxin in nucleic acid metabolism. Evans and Ray's criticism was that cell elongation is too fast, the half-life of mRNAs too long, and transcription/translation too slow in plants in order for the products of auxin-induced genes to accumulate and regulate cell elongation.

Accordingly, "not much was done between 1969 and 1977," says Joe Key of the University of Georgia. In 1977, however, following the development of more sophisticated recombinant-DNA technology, work in this area of research was revived. "We were able to ask more specific questions about the numbers and kinds of genes the expression of which were affected by auxin," says Key.

In more recent years, Key's group, along with Tom Guilfoyle of the University of Missouri and Athanasios Theolopoulos in the USDA's Plant Gene Expression Center in Albany, California, have defined genes that encode a group of auxin-regulated RNAs. Key says that no information is available on the sequence of events that occurs between the entry of auxin into a cell and the enhanced expression of these genes. "But there cannot be many events because the plant response time is very short," he adds.

Early in 1989, Guilfoyle provided the strongest evidence to date correlating auxin-responsive genes with a biological response: cell elongation attributed to auxin. He observed changes in the distribution of auxin-regulated RNAs in a soybean hypocotyl, a part of the axis of the plant embryo that is associated with bending and elongating in response to gravity. When placed in a horizontal position, the hypocotyl bends upward. Prior to that morphological response, the expression of these genes decreases rapidly on the upper side of the horizontal soybean hypocotyl and dramatically increases on the lower side, which is the side that elongates in response to gravity. Thus, biochemical evidence was gathered for the simply stated idea, suggested decades ago, that auxin affects cell elongation.

"It is obvious that very little is known about how auxin specifically regulates gene expression or about the role of gene expression in auxin action," says Key. "However, many technologies are now in place to permit an analysis of the relevant phenomena."

"Sorting out the cascade of plant hormones and the genes that they regulate will take some time," adds Howell. "But for the first time in many years, plant biology is on the fast track." •

The National Science Foundation contributes to the support of the research discussed in this article principally through its Genetics and Developmental biology programs.