Oligosaccharins

Fragments of the plant cell wall have been discovered that serve as regulatory molecules. They help to control such functions as growth, development, reproduction and defense against disease

by Peter Albersheim and Alan G. Darvill

Every higher plant is made up of a multitude of cells, all of which have the same complement of genes. How then can different cells have different functions and be shaped differently, so that plants have roots, stems, leaves, flowers and fruits? The answer is that only a small subset of the genes in a particular kind of cell are expressed, or turned on, at a given time. A complex system of chemical messengers activates the genes responsible for forming flowers in some cells, for example, and the genes responsible for root formation in other cells.

The chemical messengers in plants are called hormones or regulatory molecules. Five major hormones have been identified: auxin, abscisic acid, cytokinin, ethylene and gibberellin. In our studies we have identified a new class of regulatory molecules in plants. We call them oligosaccharins. Each of them appears to deliver a message regulating a particular plant function. The functions include defense against disease, growth, and differentiation in the course of development: whether to form roots, stems, leaves or flowers and fruits.

Unlike the oligosaccharins, the five well-known plant hormones are pleiotropic rather than specific, that is, each has more than one effect on the growth and development of plants. Auxin, for instance, stimulates the rate of cell elongation, causes shoots to grow up and roots to grow down and inhibits the growth of lateral shoots. Auxin also causes the plant to produce a second hormone (ethylene), to develop a vascular system and to form lateral roots. The other four well-known plant hormones have a similarly complex array of functions. Indeed, they have so many simultaneous effects, some of them beneficial and others harmful, that they are not of great commercial value in agriculture. For example, the same hormone can have both a beneficial effect such as increased mobilization of food reserves and a harmful effect such as loss of leaves.

The pleiotropy of the five well-studied plant hormones is somewhat analogous to that of certain hormones in animals that stimulate a gland to release many other hormones. For example, hormones from the hypothalamus in the brain stimulate the anterior lobe of the pituitary gland to synthesize and release many different hormones, one of which is corticotropin. Corticotropin in turn stimulates the release of a number of different hormones from the adrenal cortex. The other hormones released from the anterior pituitary also have specific effects on target organs all over the body. One hormone stimulates the thyroid gland, for example, another the ovarian follicle cells and so forth. In other words, there is a hierarchy of hormones.

We think such a hierarchy may also exist in plants. Oligosaccharins are fragments of the cell wall. They are released from the cell wall by enzymes; different enzymes release different oligosaccharins. There are indications that pleiotropic plant hormones such as auxin and gibberellin may actually function by activating the enzymes that release these other, more specific chemical messengers from the cell wall.

Our discovery of oligosaccharins was the direct result of the fact that for many years our laboratory at the University of Colorado at Boulder was simultaneously engaged in two research projects we thought were unrelated. The first project was an effort to elucidate the structure of the plant cell wall. It was the remarkable complexity of the cell-wall components that first suggested their role might be more than a structural one. The second line of research was a study of the molecules involved in a plant's defense against disease. This research pointed directly to the possibility that the cell wall might act as a pseudo-gland: a repository of precursors of a class of regulatory molecules that, on being released, are capable of controlling a number of plant functions.

Plant cells are characterized by a distinctive semirigid envelope, the cell wall. One of the wall's functions is mechanical: it gives the cell, and thus ultimately the plant, strength and form. It lies outside the cell's plasma membrane, which defines the boundary of the cell's cytoplasm chemically. The cell wall is permeable to most molecules; the membrane's permeability is highly selective, enabling it to control the entry of metabolites into the cell and the transport out of the cell of cell-wall components and other molecules synthesized in the cytoplasm. The "primary" wall of a young cell is thin, and it enlarges rapidly. The "secondary" wall of a mature, nongrowing cell is thicker and may assume a shape more distinctive than that of the generally boxlike primary wall.

We study primary walls, which consist almost entirely (about 90 percent) of polysaccharides, large molecules made up of interconnected simple sugars called monosaccharides. (Proteins account for the remaining 10 percent of the wall.) Most cell-wall monosaccharides are either pentoses or hexoses: they have five or six carbon atoms, along with hydrogens and oxygens.

D-glucose is the most abundant sugar in cell walls; indeed, it is the most abundant sugar in nature. (The names of monosaccharides are often prefixed by a D or an L. The prefix identifies the orientation of chemical groups linked to one of the carbon atoms—carbon 5 of a hexose. Most sugars in primary cell walls exist in only one of the two configurations, and so a monosaccharide can generally be referred to without its prefix.) The structure of glucose is typical of the structure of all the monosaccharides found in cell walls.

In aqueous solution glucose can exist as an open chain of six carbon atoms, but far more often it takes the form of a ring in which an oxygen atom connects carbon 1 and carbon 5. In the ring form carbon 1 has a hydrogen atom and a hydroxyl group (OH) attached. There are two versions of the ring because the hydrogen and the hydroxyl can be oriented in either of two ways, designated alpha and beta. In solution the open-chain form and the alpha and beta ring forms interconvert continually.

In polysaccharides and oligosaccharides (small polysaccharides containing from two to perhaps 15 monosaccharides) the monosaccharides are linked to one another by the distinctive glycosidic bond. This covalent bond (a bond in which atoms share pairs of electrons) is formed when carbon 1 of a sugar in the ring configuration is linked to a different carbon atom of another sugar. A molecule of water is extracted from the hydroxyl groups attached to both carbon atoms, leaving a single oxygen atom connecting the two sugar units.

Because the oxygen and hydrogen atoms attached to the carbon 1 of a sugar can have either of two orientations, alpha or beta, there are alpha and beta glycosidic bonds. Although the difference between the two kinds of bond seems small, the same two sugars yield radically different disaccharides depending on whether they are alpha- or beta-linked. For example, two glucose molecules linked by an alpha glycosidic bond from carbon 1 to carbon 4 form the disaccharide maltose; many glucose molecules linked in this way make the polysaccharide starch. Two glucose molecules linked by a beta glycosidic bond at the same positions, however, yield cellobiose; a long chain of glucose molecules thus connected makes the polysaccharide cellulose. Cellulose is identical with starch in chemical composition, but its properties are very different.

Cellulose is the best-known and the best-understood of the polysaccharides of the cell wall. It is a glucan chain (a chain consisting only of glucose units) linked by beta glycosidic bonds between carbon 1 and carbon 4. The chains are linear, and in the primary wall they aggregate to form fibers made up of about 40 chains each. These cellulose fibers are primarily responsible for the strength of the cell. They are embedded, like the steel reinforcing rods in concrete, in a matrix of other molecules, most of which are also polysaccharides.

The matrix of a typical primary cell wall is composed of at least eight polysaccharides. Six of them have been well defined. They are homogalacturonan, rhamnogalacturonan I, rhamnogalacturonan II, xyloglucan, arabinogalactan and glucuronarabinoxylan. Each is named for its chief monosaccharide constituents. For example, the two sugars that are most prevalent in rhamnogalacturonan II are rhamnose and galacturonic acid, although the polysaccharide includes at least eight other sugars.

Until quite recently the matrix polysaccharides were assumed to be more or less like cellulose in structure: chemically simple sets of sugar molecules bonded together in regular, predictable, easily detected patterns. In fact, however, they are vastly more complex. Whereas cellulose is made up of identical sugars, all bonded in the same way, the matrix polysaccharides are instead made up of two or more kinds of sugars connected by several kinds of glycosidic bond. Two molecules of a six-carbon sugar such as glucose can be joined in 64 distinct ways; three different sugar molecules can be joined in more than 1,000 ways! Since a single polysaccharide can incorporate hundreds or even thousands of

FLOWERS grow from a sliver of a tobacco-plant stem maintained in a liquid culture medium. Each flower has an ovary in the center, surrounded by five anthers and a number of poorly developed petals and sepals. The authors and their colleagues induced the tobacco-stem sliver, or "explant," to develop flowers by adding to the medium newly discovered substances called oligosaccharins. These are oligosaccharides (short chains of sugar molecules) that are cleaved from the plant cell wall and act as hormones, or regulatory molecules. Different oligosaccharins have been shown to exert different influences on plant development (*see illustration on page 64*).

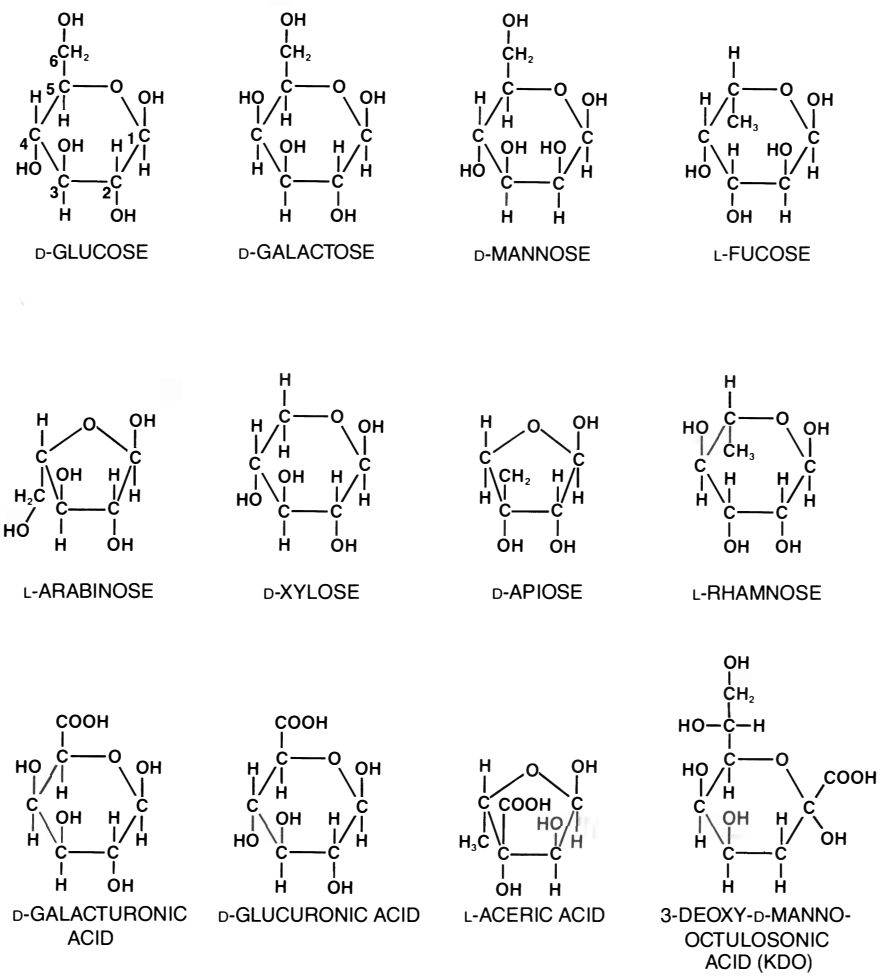
monosaccharides, the number of different potential arrangements is staggering; one cannot guess how many are allowed by the constraints of the real world. The structural complexity of wall polysaccharides is often increased by the presence of nonsugar groups such as methyl ethers and methyl or acetyl esters.

We came to understand the extent and significance of this complexity with the help of new technology. We degraded the cell wall with various enzymes that release specific polysaccharides for analysis and we analyzed the polysaccharides with advanced, computer-assisted chromatographic and spectrometric instrumentation. These techniques have revealed structural features that were previously inaccessible. For example, we have recently uncovered two "new" plant cell-wall sugars: aceric acid and KDO.

Both of them were identified as minor components of rhamnogalacturonan II, whose structure illustrates the extreme complexity of the cell-wall polysaccharides. Rhamnogalacturonan II accounts for approximately 3 percent of the primary cell wall of dicotyledons (broad-leaf plants with a netted pattern of veins); it is present in smaller amounts in both monocotyledons (narrow-leaf plants with parallel veins) and gymnosperms (which include the conifers).

We isolated rhamnogalacturonan II by treating the primary cell wall of cultured sycamore cells with a specific polysaccharide-cleaving enzyme. It makes soluble approximately 15 percent of the cell wall, including all the rhamnogalacturonan II. We purified the rhamnogalacturonan II to homogeneity and found it is composed of 10 different sugars linked in at least 20 different ways—by alpha and beta bonds and through different carbon atoms. We have established that rhamnogalacturonan II is made up of some 65 monosaccharides. So far we have characterized five different oligosaccharides, which together account for most of the 65. We are working to characterize the rest of the components. Then we shall have to learn how they are all fitted together in order to determine the complete structure of this highly complex molecule.

Rhamnogalacturonan II appears to be more complex than any polysaccharide whose structure has been established. Why should it turn out to be a component of every plant cell wall that has been studied? Why do cell-wall matrix polysaccharides in general not have a simple structure, more like that of cellulose? Why do they apparently contain far more chemical information than would be required simply to hold a plant upright?

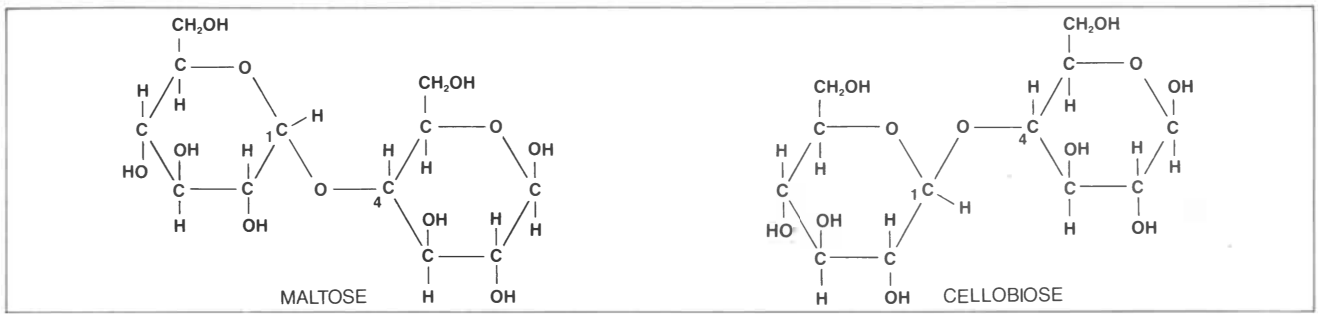


TWELVE SUGARS (monosaccharides) known to be components of the primary cell wall of plants are depicted schematically, with the ring compressed into a single plane. The hexoses (six-carbon molecules) D-glucose, D-galactose and D-mannose are stereoisomers, differing only in the arrangement of the groups attached to their carbon atoms. L-fucose and L-rhamnose lack a hydroxyl (OH) group at carbon 6. The pentoses L-arabinose, D-xylose and D-apiose have only five carbon atoms; 3-deoxy-D-manno-octulosonic acid (KDO) has eight and all the others have six. D-galacturonic acid, D-glucuronic acid, L-aceric acid and KDO carry carboxyl (COOH) groups and hence are acidic sugars. The D- and L- refer to the arrangement of chemical groups linked to carbon 5 of hexoses, carbon 4 of pentoses and carbon 6 of KDO. Only one such configuration (D or L) is found for each sugar in primary cell walls. Studies with new computer-assisted instrumentation and specific enzymes have only recently revealed that KDO and L-aceric acid are present in the primary wall.

Possible answers emerged from our research on the chemical basis of disease resistance in plants. A plant defends itself against fungal or bacterial pathogens by producing antibiotics, called phytoalexins, at the site of infection. Antibiotics are not proteins, and so they are not the direct product of gene expression. What needs to be expressed to make an antibiotic is the set of genes encoding the enzymes that catalyze antibiotic synthesis. Some chemical message has to deliver the signal for expression.

What is that message? We asked: Is there something in the infecting microorganism that signals a plant to make an antibiotic? We chose to work with a fungus that attacks soybeans because James A. Frank and Jack D. Paxton of the University of Illinois at Urbana-Champaign had developed a method for assaying the level of antibiotic synthesis in response to this particular fungal infection.

We learned that what plants recognize is the presence of a fungal oligosaccharide: a fragment released from a polysaccharide that is a structural component of the fungal cell wall. It was quite a surprise. Oligosaccharides, the short chains of sugars we had worked with for years, were not known to be able to carry information and so serve as chemical messages. And yet adding to plant cells a mixture of oligosaccharides (which were released by the random cleavage of a fungal cell wall with acid) could make the plant cells synthesize the enzymes that



GLYCOSIDIC BOND is the characteristic linkage between sugars in polysaccharides. The carbon 1 of a monosaccharide is linked to a carbon atom of a second sugar unit. A molecule of water is extracted from the hydroxyl (OH) groups attached to both carbons, leaving a single oxygen atom connecting the two sugar units. There are two versions of the glycosidic bond, alpha and beta, because the hydrogen atom and the glycosidic oxygen atom attached to carbon 1 can be oriented in two ways. Linking two sugar molecules at the same carbons but with different glycosidic bonds yields very different results. Two glucose molecules linked by an alpha glycosidic bond from the carbon 1 of the first molecule to the carbon 4 of a second one (*left*) form the disaccharide maltose; many glucose molecules linked in this way form starch. A beta glycosidic bond linking two glucose molecules from carbon 1 to carbon 4 produces the disaccharide cellobiose (*right*) instead of maltose. A chain of glucose molecules linked by such beta glycosidic bonds constitutes cellulose.

catalyze antibiotic synthesis. The oligosaccharides could clearly be considered regulatory molecules.

We set out to determine the structure of the smallest piece of the fungal cell wall that would cause plant cells to synthesize antibiotics. At first the problem did not seem too difficult because the cell-wall polysaccharide we were working with consisted only of a chain of glucose. This glucan chain turned out to be an intricately branched one, however, and so the project proved to be far more challenging than we had expected.

Cleavage of the polysaccharide had yielded a complex mixture of glucose-containing oligosaccharides of different sizes. We separated the oligosaccharides according to the number of glucose units in each and determined that the smallest fragment capable of stimulating the synthesis of antibiotics in plants was a hepta-beta-glucoside: an oligosaccharide made up of seven glucose units interconnected by beta-glycosidic bonds. That finding did not exactly settle the matter, however, because there were more than 300 differently arranged hepta-beta-glucosides in the fragments of the glucan! Separating the one active form from the more than 300 inactive forms proved to be an enormously difficult task because they were chemically so similar.

It was in 1974 that we initiated the effort to purify the active oligoglucoside and almost 10 years later that we succeeded—and then only because there had been tremendous advances in analytical instrumentation. The task of isolating one pure, active heptaglucoside and five pure, inactive heptaglucosides was successfully completed in 1983 by Janice K. Sharp, a graduate student in our laboratory. She ended up with only a minute amount of each heptaglucoside. In 1974 we had expected to need a full gram of pure heptaglucoside to decipher a structure. By 1983 the technology was so improved that the 50 micrograms (50 millionths of a gram) of each heptaglucoside Sharp isolated was enough to enable us to determine their structure.

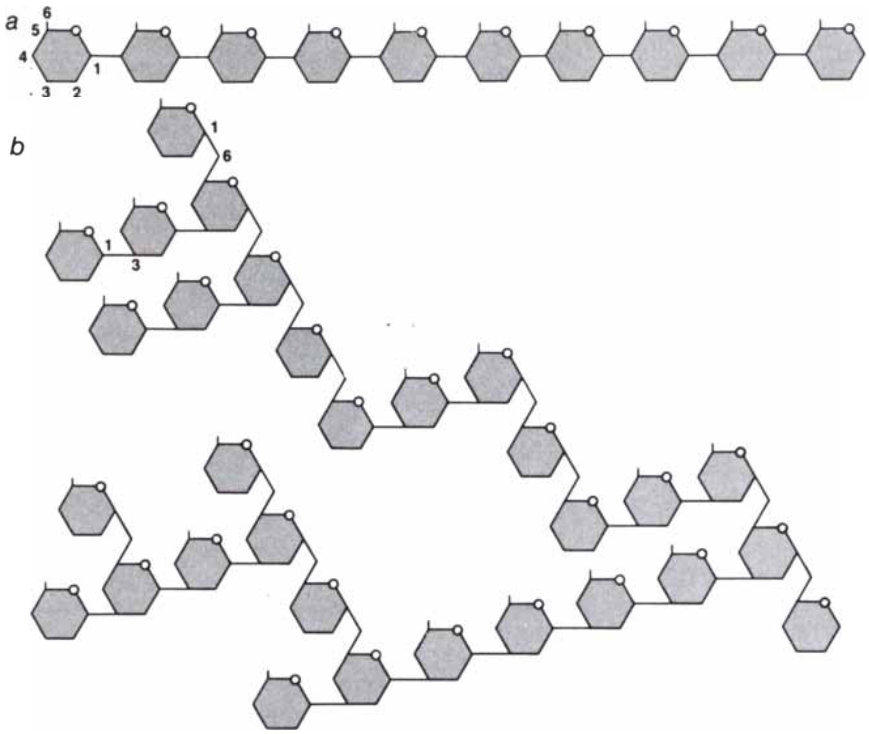
The results established that a plant recognizes and responds to a remarkably specific oligosaccharide structure. The active form—the oligosaccharin—and the inactive forms of the heptaglucoside are very similar. They differ only in the positions at which the two side chains (each a single glucose unit) attach to the backbone (a chain of five glucose units linked by beta-glycosidic bonds). The side-chain positions determine which of the heptaglucosides is active [*see bottom illustration on next page*]. The presence of only a billionth (10^{-9}) of a gram of the active heptaglucoside is enough to activate the synthesis of messenger-RNA molecules from the DNA of the enzyme genes, and so to initiate antibiotic production. The antibiotic functions to stop the growth of the fungus from which the heptaglucoside originated.

The next obvious step was to rephrase the question we had asked about fungi and ask: What is it in bacteria that causes plants to make antibiotics at the site of infection? Michael G. Hahn, another graduate student, studied the effect of several bacterial species on soybeans and got a striking answer. He found that in the case of bacterial infection the message is not in the pathogen's cell wall but in the wall of the plant cells. The presence of the bacteria somehow brought about the release of an oligosaccharide from one of the plant's own cell-wall polysaccharides. The released oligosaccharide caused nearby plant cells to make antibiotics. The active oligosaccharide turned out to be part of a pectic polysaccharide of the plant cell wall. ("Pectic" means rich in galacturonic acid, another of the simple sugars.) Eugene A. Nothnagel, a postdoctoral research associate, found the active oligosaccharide was an oligogalacturonide: a simple linear array of galacturonic acid units.

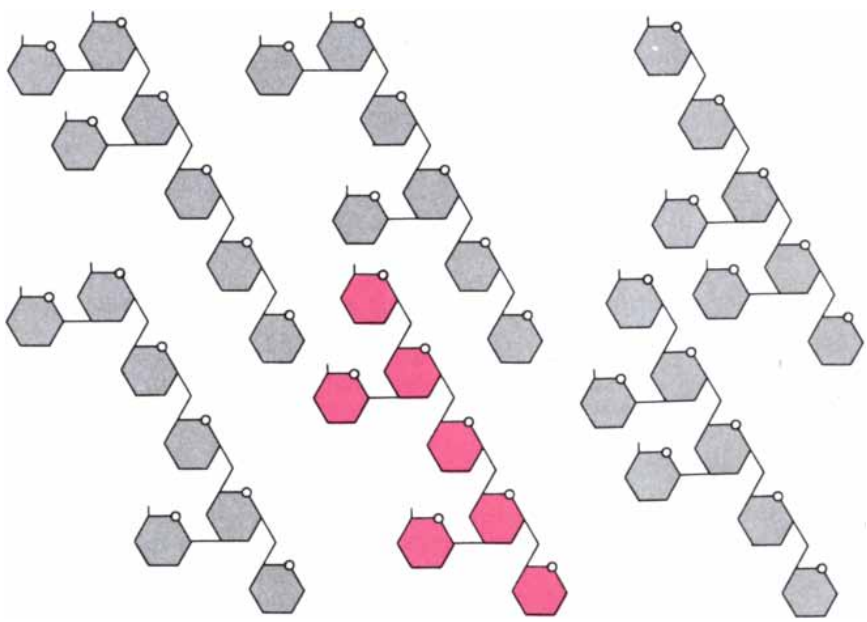
Charles A. West and his co-workers at the University of California at Los Angeles confirmed that an oligogalacturonide from the plant cell wall can stimulate plant cells to make antibiotics. They went on to describe one mechanism for the release of this oligosaccharin. In the case of a fungus that attacks castor-bean plants, an enzyme secreted by the fungus releases the oligogalacturonide from the cell walls of the plant. The oligogalacturonide—an oligosaccharin—stimulates the plant cells to make antibiotics. Keith R. Davis, another graduate student, discovered a related mechanism when he purified an enzyme from a bacterial plant pathogen, *Erwinia carotovora*. Davis found the bacterial enzyme releases an oligogalacturonide from the plant cell wall, in soybeans and in the other plant species examined, that stimulates soybeans to make antibiotics.

Still another variation was discovered by Gary D. Lyon, a visiting scientist. He learned that when certain plant cells are damaged, the cells themselves produce an enzyme that releases oligosaccharides (presumably oligogalacturonides) from nearby cell walls, and that these oligosaccharides stimulate plant tissue to synthesize antibiotics. In other words, whether a bacterium, a fungus or a virus attacks and damages the cells of a plant, the plant can respond and make antibiotics; it need not "see" an enzyme supplied by the microbe.

Several different mechanisms, then, appear to effect the same result: the



GLUCOSE UNITS can be linked in many ways. The structures of parts of two beta glucans (molecules made up solely of glucose units linked by beta glycosidic bonds) are illustrated. All the glucose units (*hexagons*) are in the six-atom pyranose ring form and all are glycosidically linked from carbon 1 of the first glucose to carbon 3, 4 or 6 of the next glucose unit. When a beta glucan is arranged in a linear manner, with carbon 1 linked to carbon 4 of the next unit, cellulose is formed (*top*). A very different kind of polysaccharide is formed when carbon 1 of the first glucose is linked to carbon 3 or 6 of the next one (*bottom*). The branched arrangement and varying glycosidic linkages make this beta glucan far more complex than cellulose. The complexity is typical of polysaccharides cleaved to make oligosaccharins.



FIRST OLIGOSACCHARIN identified by the authors and their colleagues was a heptaglucoside, or seven-glucose chain (*color*), isolated from the cell wall of a fungus that attacks soybeans. It differs from a number of inactive cell-wall heptaglucosides only in the positions at which the two side chains (each a single glucose unit) are linked to the backbone (a chain of five beta-glycosidically linked glucose units). The active heptaglucoside—the oligosaccharin—is released from the fungal cell wall by an enzyme present in the host plant. Applied to soybean cells, minute quantities of the heptaglucoside oligosaccharin can activate the genes responsible for the synthesis of antibiotics that can inhibit the growth of the fungus. The inactive heptaglucosides, in any amount, never turn on the genes responsible for antibiotic synthesis, and they do not interfere with the ability of the oligosaccharin to do so.

release of an oligosaccharide from its cell-wall polysaccharide to serve as an oligosaccharin that stimulates the production of antibiotics. In our original experiments with fungi (we eventually learned) it was an enzyme from the host plant that released the heptaglucoside oligosaccharin from the fungal cell wall. In other cases it is an enzyme secreted by the disease agent, whether fungal or bacterial, that releases an oligogalacturonide oligosaccharin from the host plant's cell wall. And when a plant cell is injured, the plant cell itself secretes an enzyme that releases the oligosaccharin from the plant's own cell walls.

Davis and Brian Hodgson, who was a visiting scientist in our laboratory, have shown that two of these mechanisms may act together. When the heptaglucoside (released from the fungal cell wall by an enzyme in the plant) and the oligogalacturonide (released from the plant cell wall by an enzyme in either the pathogen or the plant) are both present, there is a synergistic effect: it takes much less of each substance working together to activate the synthesis of the antibiotic than would be required if one of the oligosaccharins were acting alone.

Having established that a fungal heptaglucoside and a plant oligogalacturonide can regulate gene expression in plant cells to generate antibiotic production, we searched for other fragments that might defend plants against insect pests. Clarence A. Ryan, Jr., of Washington State University and his colleagues had shown how plants defend themselves against certain insect enzymes (proteinases) that digest plant proteins. The plant cells synthesize inhibitors of the proteinases when the cells are triggered to do so by a chemical message Ryan called the proteinase-inhibitor inducing factor (PIIF). We collaborated with Ryan's group to establish that PIIF indeed is an oligosaccharin cleaved from a polysaccharide of the plant cell wall. Ryan and his colleagues then showed that an oligogalacturonide that stimulates synthesis of the proteinase inhibitor is similar to one shown earlier to stimulate antibiotic synthesis. It appears that oligogalacturonides can act differently, depending on what plant cells they interact with, to turn on the appropriate defense response.

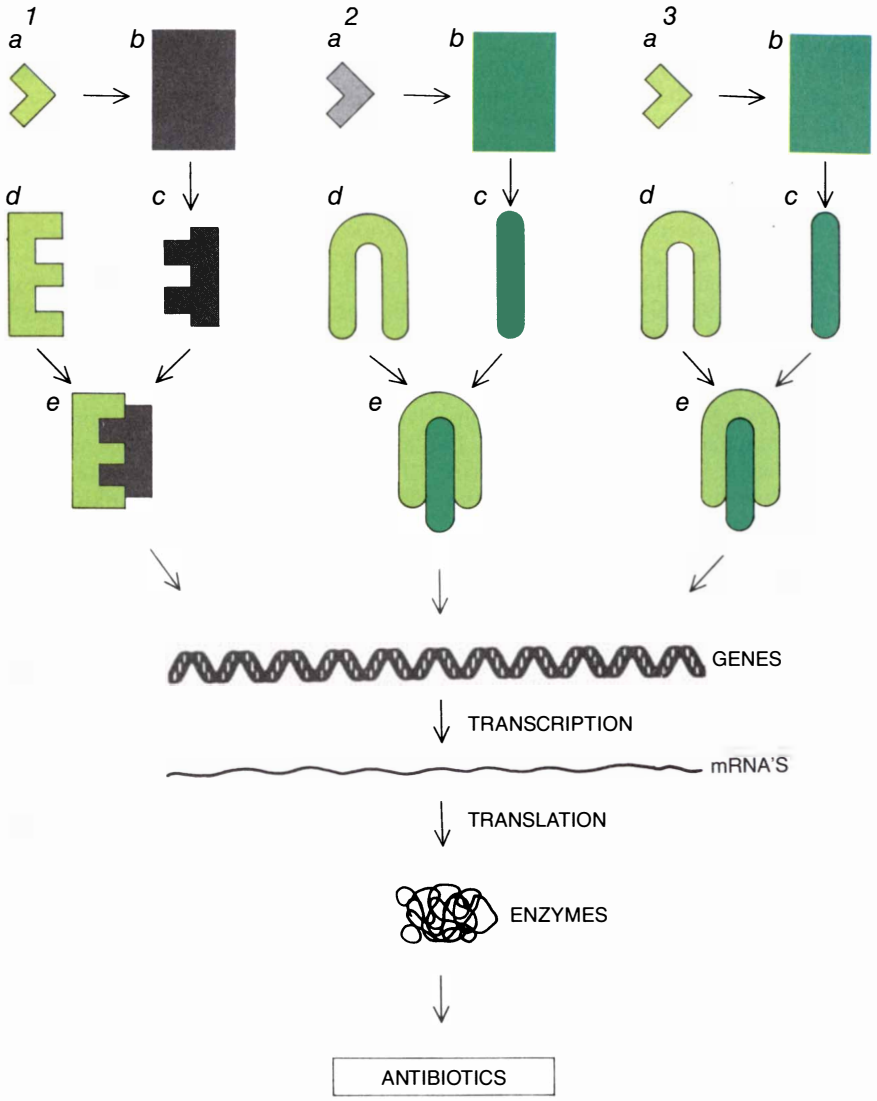
A particularly important and widely observed defense response of plants is the self-sacrifice of the first few cells of a plant that come in contact with an invading microbe. This "hypersensitive resistance response" is thought to somehow impede invading microbes long enough for other defense mecha-

nisms (such as the accumulation of antibiotics) to take effect.

We speculated that an invading microorganism might bring about the release of an oligosaccharin, from cell walls in the neighborhood of the infection, that is recognized by plant cells as a signal to undergo hypersensitive death. In support of our hypothesis Noboru Yamazaki, a visiting scientist, and we were able to isolate from the walls of sycamore cells an oligosaccharin that kills sycamore cells. Steven H. Doares, a graduate student, found that the sycamore oligosaccharins can kill not only sycamore cells but also maize cells, and that oligosaccharins from maize cell walls kill sycamore cells as well as maize cells. These results and others indicate that the cellular suicide may well be triggered by the cell's recognition of oligosaccharins released from neighboring cell walls in response to an invading microbe. The oligosaccharin that induces cell death is not an oligogalacturonide but some other fragment having a more specific function.

Having identified oligosaccharins able to activate defense responses in plants, we considered the possibility that similar molecules help to regulate other functions of plants, such as growth, development and reproduction. Was it possible that (at least in some cases) classical plant-growth regulators such as auxin and gibberellin work indirectly, by activating specific enzymes that release oligosaccharins—which in turn directly regulate many of the normal physiological processes of plants?

The ability of auxin (and synthetic auxins such as 2,4-D) to stimulate the growth of pea-seedling stem segments has been studied for many years. William S. York and we studied the ability of oligosaccharides from the sycamore cell wall to modulate auxin's effect on the rate of growth of pea-stem segments. We found that mixtures of oligosaccharides extracted from sycamore cell-wall polysaccharides could inhibit auxin-stimulated growth in pea-stem segments maintained in a culture dish. It took about three years to isolate a highly purified, active oligosaccharin fraction from xyloglucan, a polysaccharide present in the primary walls of plant cells. The fraction strongly inhibits auxin-stimulated growth of pea stems. We believe the active oligosaccharin is either a nonasaccharide (a nine-sugar fragment) or another fragment very closely related to the nonasaccharide. The concentration of the oligosaccharin required to inhibit auxin-induced growth is about 100 times lower than the concentration of auxin required to stimulate growth.



SYNTHESIS OF ANTIBIOTICS by plants is triggered by oligosaccharins. Three mechanisms are known by which an oligosaccharin can be released from a cell wall. In some fungal infections (*1*) an enzyme from the infected plant (*a*) cleaves from the fungal cell wall (*b*) a heptaglucoside fragment (*c*). The fungal heptaglucoside is an oligosaccharin. In other cases (*2*) the fungal or bacterial pathogen supplies an enzyme that cleaves the infected plant's cell walls, releasing an oligogalacturonide oligosaccharin (*c*). Finally, in some cases a plant that is damaged in any of several ways (*3*) itself supplies an enzyme that cleaves the oligogalacturonide from its own cell walls. In all three cases the oligosaccharin presumably combines with a receptor in the plant (*d*) to form an activated signal molecule (*e*). The signal causes a number of plant genes to be transcribed into messenger RNA's, which in turn are translated into enzymes. The enzymes then catalyze the synthesis of various antibiotics.

This work dovetailed neatly with findings reported about 10 years ago by Gordon A. Maclachlan of McGill University and his colleagues. They demonstrated that treating pea stems with auxin not only stimulates growth but also increases about fiftyfold the activity of a particular enzyme in the wall of the pea-stem cells. They later found that the enzyme cleaves xyloglucan into its oligosaccharide components, which are predominantly nona- and heptasaccharide fragments. We have now found that the nonasaccharide fragment of xyloglucan (or a closely related oligosaccharide) released by the cleavage enzyme inhibits auxin-induced growth. In other words, auxin (which in general stimulates pea stems to grow) also activates the enzyme that releases a cell-wall oligosaccharin acting to inhibit the auxin-induced growth. Such "feedback" modulators of hormonal activity are well known in animal systems but had not been shown to operate in plants.

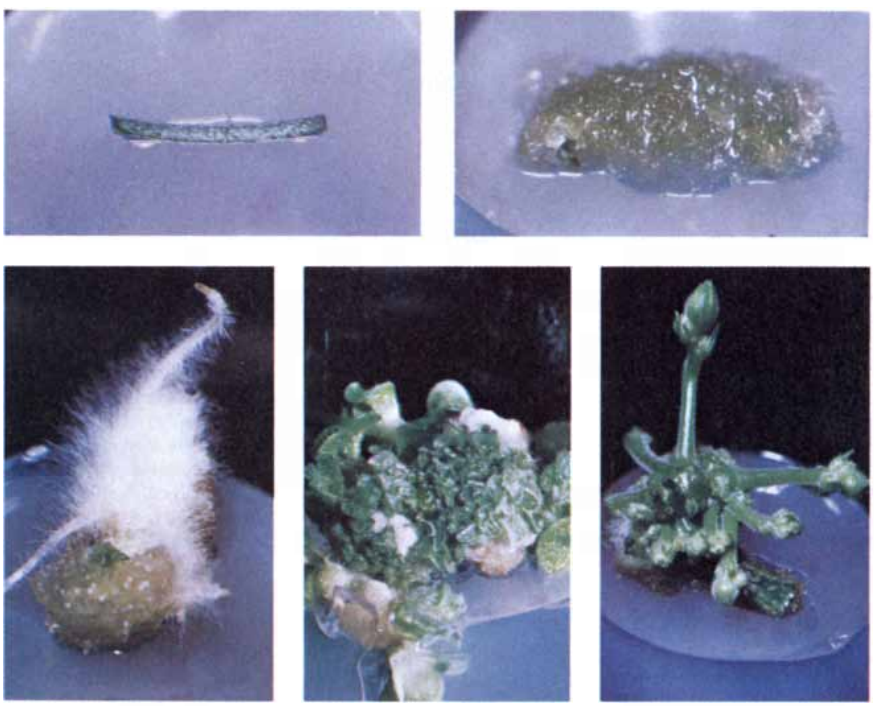
We assume that auxin increases the activity of the polysaccharide-cleaving enzyme by making more of it—by activating the genes encoding it. We think

that when auxin thus increases the amount of the oligosaccharin inhibiting auxin-induced growth, the oligosaccharin may be transported down the plant stem to inhibit the growth of lateral buds. It is well known that cutting off the apical bud at the tip of a plant makes the plant bushier. It has been thought that removing the bud removes the source of auxin, which has been assumed to be directly responsible for apical dominance, or the inhibition of lateral growth. We propose instead that removing the source of auxin actually decreases the amount of the enzyme that releases the inhibiting oligosaccharin from xyloglucan. If we are right, it is the oligosaccharin (which, we have shown, inhibits the growth of stem segments) that directly inhibits lateral bud growth. Experiments are now under way to find out whether the oligosaccharin makes a plant grow tall and thin by inhibiting the growth of lateral buds.

Might oligosaccharins have roles not only in defense responses and the control of growth and shape but also in regulating organ development (morphogenesis) and reproduction? To determine their role in morphogenesis and reproduction we wanted to test their ability to induce plant tissue growing in a culture medium to form particular organs. Kiem Tran Thanh Van and her colleagues at the Laboratoire du Phytotron at Gif-sur-Yvette in France have developed an elegant tissue-culture system for testing the effect of factors in a culture medium on the growth of thin strips of tobacco-plant flower stems. They floated thin tobacco "explants" on liquid culture mediums containing salts and glucose (as a source of energy). They added precisely predetermined amounts of auxin and cytokinin and adjusted the acidity of the culture medium. In a series of experiments they found that the ratio of auxin to cytokinin and the acidity of the culture medium determined whether the explants grew to form undifferentiated callus or differentiated to form either flowers, vegetative buds or roots.

We hypothesized that the actual effect of the auxin, cytokinin and level of acidity is to influence the release and modification of an array of chemical messages, each of which regulates the biochemical reactions responsible for a particular developmental program. Tran Thanh Van, Patrick Toubart and Alain Cousson at Gif-sur-Yvette collaborated with David J. Gollin, Paulanne Chelf and us to test this possibility.

We added mixtures of oligosaccharides isolated from sycamore cells to the tobacco-explant culture medium to see whether the mixtures included oligosaccharins able to influence organ development. There were several different mixtures of oligosaccharides, including one we had earlier shown to inhibit flowering and promote vegetative (nonreproductive) growth in duckweed. The same mixture that inhibited flowering in duckweed was found to both inhibit flowering and stimulate prolific vegetative budding in the tobacco explants. The oligosaccharins were effective in impressively low amounts, from 100 to 1,000 times less than the amounts of auxin and cytokinin present in the media. In the presence of a different mixture explants that formed vegetative buds in the absence of added oligosaccharides formed flowers. Still another oligosaccharide mixture caused explants that would ordinarily have formed vegetative buds or callus to form prolific roots instead.



EFFECT OF OLIGOSACCHARINS on morphogenesis was tested with tobacco explants by Kiem Tran Thanh Van and her colleagues. The stem of a flower of a tobacco plant is cut off and strips composed of the epidermis (one cell layer), subepidermis (one cell layer) and parenchyma (from one cell layer to three layers) are removed. The strips are one or two millimeters wide and 10 millimeters long (*top left*). Some 20 such explants are floated in a liquid medium containing glucose (as an energy source) and salts in a petri dish. The plant hormones auxin and cytokinin are added, along with various mixtures of oligosaccharins supplied by the authors. The ratio of auxin to cytokinin, the acidity of the culture medium and the particular mix of oligosaccharins determine the developmental response. As is shown by these photographs (which are of single explants grown on solid agar), an explant may, under the influence of various oligosaccharins, form an undifferentiated callus (*top right*), roots (*bottom left*), vegetative shoots and leaves (*bottom middle*) or floral shoots (*bottom right*).

The ability of oligosaccharins to regulate organ development in tobacco explants is compelling evidence for the possibility that these cell-wall fragments act as regulators of morphogenesis and reproduction in plants. The results of the experiments we have described, together with our original findings on the complexity of cell-wall polysaccharides, support our theory that plant cell walls are the repository of a large number of specific oligosaccharins. The oligosaccharins may represent another tier in a hierarchical hormone system. They appear to regulate not only the activation of defense mechanisms but also aspects of plant growth, morphogenesis and reproduction. One day it may be possible to spray specific oligosaccharins (or analogues of oligosaccharins) on plants (or to manipulate the genes controlling the release and metabolism of oligosaccharins) to tell plants to flower or to form seeds and fruits or tubers, to become resistant to a disease or an insect, to drop their fruit, to grow faster or to become bushier. Oligosaccharins should eventually have a significant impact on agricultural yields.