Looking at Lectins: Do They Function in Recognition Processes?

Cell biologists have long found the plant proteins called lectins to be useful tools for probing the structure of cell membranes. But lectins did not evolve for the convenience of cell biologists, and, until recently, no one knew what function the proteins perform for the plants in which they occur. Now, although many questions remain unanswered, there is growing evidence that lectins participate in the recognition processes between plants and bacteria. Receiving particular attention is the role played by the proteins in the process by which nitrogen-fixing bacteria of the genus *Rhizobium* recognize their legume hosts. Lectins may also be involved in the defense mechanisms that plants have against pathogenic bacteria.

Moreover, the distribution of lectins—or at least lectin-like proteins—may be wider than generally thought. Recent research has uncovered such proteins even in mammals and birds; and here, too, they appear to function in recognition processes.

The association between legumes and their symbiotic nitrogen-fixers is very specific. Even though many kinds of rhizobia and other microorganisms live in the soil around the plant roots, each species of legume is usually infected by only one rhizobial species, and, conversely, most rhizobia infect only one kind of legume. Investigators now think that the basis for this specificity may reside in the capacity of the bacterial cell to recognize and bind to some component found in the roots of the right plant; the emerging view is that a lectin might well be that component.

Lectins are characterized by their ability to recognize and bind to specific sugars, including those in the complex carbohydrate-containing materials found on cell surfaces. Many lectins have two or more binding sites and can thus bind to more than one cell, form bridges between the cells, and cause them to clump. For example, the capacity to aggregate red blood cells, which have many surface carbohydrates, is a common criterion used to identify lectins.

Bacterial cells also have sugar-containing substances on their surfaces, which may differ from species to species. Much of the evidence implicating lectins in the establishment of symbiotic nitrogen-fixing relationships depends on demonstrations that a lectin from a particular legume binds only to the corresponding rhizobial species and not to bacteria that infect other legumes.

One of the first such observations was made in 1974 by Edwin Schmidt and B. Ben Bohlool of the University of Minnesota. They showed that soybean lectin labeled with a fluorescent stain binds to 22 of 25 strains of *Rhizobium japonicum*, which infects soybeans, but not to any of 23 strains from five species of rhizobia that infect other legumes. More recently, W. D. Bauer and his colleagues at the Charles F. Kettering Research Laboratory performed binding experiments similar to those of the Minnesota group and obtained similar results. In addition, they showed that a sugar known to be a specific inhibitor of the binding of soybean lectin to red blood cells also inhibits binding of the lectin to *R. japonicum*.

Peter Albersheim and Jack Wolpert of the University of Colorado used a somewhat different approach; they isolated lectins from the seeds of four legumes (soybean, pea, red kidney bean, and jack bean) and lipopolysaccharides from the four corresponding rhizobial species. In all cases, the bacterial lipopolysaccharide interacted only with the lectin from the legume with which the bacterium forms a symbiotic relationship.

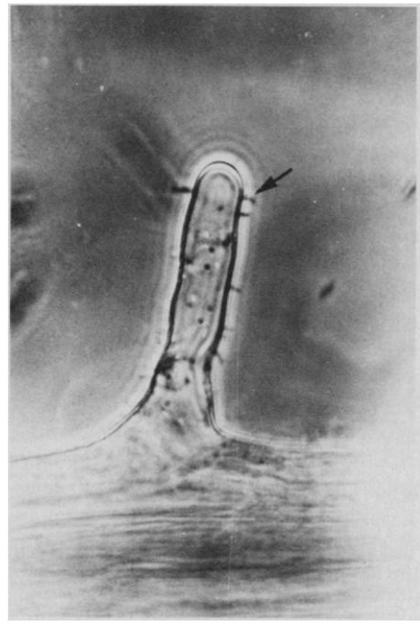

Fig. 1. Phase contrast interference micrograph of a clover root hair with attached *Rhizobium trifolii* cells (arrow). The bacterial cells attach to the root hair by one end. [Source: Frank Dazzo and Winston Brill of the University of Wisconsin]

Lectins may help to bind rhizobial bacteria to the roots of the correct legume by forming links between binding sites or receptors on the bacterial cells and on root hairs. [These small projections from the cells that form the outer layer of roots are the sites where the bacteria attach (Fig. 1).] Much of the experimental basis for this hypothesis comes from the research of Frank Dazzo and David Hubbell of the University of Florida, who were investigating the symbiotic relationship between white clover and *R. trifolii* bacteria. They showed that clover roots and infective bacteria possess a common surface antigen. An antigen is any substance that elicits the production of antibodies in animals. Antigens that are immunologically similar react with the same antibody. Dazzo and Hubbell found that antiserum prepared against clover roots also reacts with infective *R. trifolii* bacteria, and antiserums against the bacteria react with clover roots. But rhizobial strains that do not infect clover, do not bind the antiserums.

They also showed that a clover seed lectin that they named trifoliin binds to isolated *R. trifolii* antigen and to strains of the bacteria that infect clover but not to those that are noninfective. By binding to the common antigen on clover roots and on bacteria, the lectin could provide a bridge for the preferential adsorption of infective strains of *R. trifolii*.

According to Dazzo and Hubbell, the antigen prepared from *R. trifolii* is an acidic polysaccharide of high molecular weight that is composed of several sugars including 2-deoxyglucose. They have evidence that trifoliin binds specifically to this sugar component of the antigen. Moreover, the sugar itself inhibits adsorption of *R. trifolii* cells to clover roots. This is what would happen if the sugar, by combining with the lectin, either prevented binding of the bacteria or removed the lectin from the roots.

The latter may be the case. Recently, Dazzo, now working in Winston Brill's laboratory at the University of Wisconsin, demonstrated that 2-deoxyglucose prevents the binding of *R. trifolii* polysaccharide to clover roots and causes the release of a protein from intact roots. The protein is lectin-like in that it aggregates infective *R. trifolii* bacteria but not those of two rhizobial species that do not infect clover. Moreover, the aggregation is inhibited by 2-deoxyglucose. Dazzo

and Brill think that the protein may serve as the postulated bridge between the roots and the bacteria. If it is, they will have provided evidence that clover roots contain a lectin needed for bacterial attachment. One of the criticisms of much of the other research showing specific binding of lectins by bacteria or bacterial components is that the investigators used lectins prepared from seeds; the roots might not contain the same lectins.

As shown in several kinds of micrographs, one end of the rod-shaped rhizobial cells makes the connection to the root hairs when the bacteria bind to host legumes (Fig. 1). In addition, by using fluorescent-labeled antibodies against *R. japonicum*, Bohlool and Schmidt found that many strains have antigens in common that are concentrated at one end of the cell. They observed a similar localization of bound material when they treated the bacteria with fluorescent-labeled soybean lectin. Because the bacteria in culture aggregate in a head-to-head manner with the antibody-labeled ends in contact, forming rosettes or star-shaped structures, the investigators hypothesized that the same end might also bear the lectin-binding sites and be the one that attaches to legume roots.

Further analysis showed, however, that the lectin- and antibody-binding sites were at opposite ends of the bacterial cells. Schmidt and H. C. Tsien, also of the University of Minnesota, could distinguish the two ends because the compounds with high molecular weights in which the bacteria store their energy reserves accumulate in one end. The antibody-binding site could be clearly seen as a caplike structure called the extracellular polar body on that end, whereas most of the lectin-binding material is located on the other one. The extracellular polar body, which was not previously described, contains polysaccharides, lipopolysaccharides, and possibly proteins; the lectin-binding material appears to be polysaccharide secreted from the cell (exopolysaccharide).

Schmidt says that they are not discouraged by the concentration of lectin-binding material at what appears to be the "wrong" end of the bacterial cell. He points out that it is present in lesser concentrations over the entire cell and may still be involved in recognition.

This rather unexpected result points up a major unsolved problem concerning the role of lectins; that is, what is the biochemical nature of the lectin-binding site on the bacteria? The research of the Minnesota workers indicates that it may be exopolysaccharide; that of Dazzo suggests that it is capsular polysaccharide (although exopolysaccharide and the capsular material may be the same); and that of Albersheim and Wolpert indicates that it is lipopolysaccharide. An obvious explanation of the discrepancies is that different strains of bacteria may have different kinds of lectin receptors. It is also possible that one or more of the preparations was impure. In any event, a great deal of additional work will be needed to clarify this issue.

Not all investigators have found that legume lectins bind only to the corresponding rhizobial species; however, they used seed, not root lectins, and may simply have been looking at the wrong lectin.

Observations by Bauer and the Minnesota investigators that some infective strains of *R. japonicum* do not bind soybean lectin constitute another obstacle to acceptance of the hypothesis that lectins function in recognition processes. The obstacle may not be insurmountable, however. Several of the researchers have evidence that the ability of rhizobia to bind the corresponding legume lectins may vary during the growth cycle of the bacteria and with the culture conditions. For example, Bauer recently determined that the lectin-binding sites on several strains of *R. japonicum* appear and disappear during the growth cycle. Not all the strains behaved in the same manner. Dazzo and Brill have also observed variations in lectin-binding by *R. trifolii* during the growth cycle.

Moreover, rhizobial cells in culture may differ from those in the soil around legume roots or in the nitrogen-fixing nodules formed after the bacteria invade the roots. Bauer says that preliminary results in his laboratory indicate that four strains of *R. japonicum* that do not bind soybean lectin in artificial growth mediums do so when grown on soybean root surfaces or root exudate. Conversely, Schmidt has shown that strains of rhizobia that normally bind lectins when grown in culture do not have this ability in the root nodules. Thus, some strains may only appear to lack lectin receptors because they were tested under the wrong conditions.

Although most of the work on lectin function has concentrated on their interaction with symbiotic bacteria, some researchers are investigating their roles in the defenses of plants against invasion by harmful bacteria. According to Luis Sequeira of the University of Wisconsin, when tobacco leaves are inoculated with avirulent strains of *Pseudomonas solanacearum*, a pathogen of tobacco and potato plants, the bacteria rapidly attach to plant cell walls and are then enveloped by the cell walls. Virulent bacteria, however, remain free in the spaces between cells, where they multiply and spread. Sequeira hypothesizes that the avirulent strains may be attaching to lectins. He has tested 55 virulent and 34 avirulent strains of *P. solanacearum* with a potato lectin and found that all the avirulent and none of the virulent ones clump when mixed with the lectin.

Other investigators have shown that virulence for this pathogen is correlated with the presence of an extracellular polysaccharide slime. The same appears to be true for the capacity to escape clumping with lectin. After the slime is removed by washing, virulent bacteria clump when mixed with lectin; adding the slime to the avirulent forms prevents them from aggregating. Sequeira's preliminary evidence indicates that the lectin binding sites are present in the bacterial lipopolysaccharide.

Under certain conditions, the introduction of bacteria into tobacco leaves makes the whole plant resistant to subsequent infection by various pathogens including other bacterial species and even some viruses. According to Sequeira, lipopolysaccharide isolated from both *P. solanacearum* and some nonpathogens of tobacco is sufficient to induce disease resistance. The purified material attaches to cell walls in the tobacco leaves and produces cellular changes similar to those induced by binding of heat-killed cells. Sequeira speculates that, if attachment and envelopment of bacteria result from the postulated interaction between bacterial lipopolysaccharide and lectin, the same interaction may also be involved in the induction of generalized disease resistance. He is now trying to identify the smallest portion of the lipopolysaccharide molecule that will produce the effect. Sequeira says that if disease resistance can be induced with a simple molecule, development of a chemical spray to "immunize" plants against disease might one day be possible.

The word lectin is usually associated with substances obtained from plants but similar proteins also exist in a diverse group of other organisms including bacteria, cellular slime molds, sponges, and apparently even birds and mammals. In all cases, the lectins appear to function in recognition processes between cells or between cells and various carbohydrate-containing molecules.

For example, Samuel Barondes and his colleagues at the School of Medicine of the University of California at San Diego have evidence that lectins pro-

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duced by slime molds mediate the cellular aggregation that occurs during the development of these organisms. In the early stage of their life cycle, slime molds exist as amoeboid cells that, under appropriate conditions, aggregate to form a living slime that in turn forms fruiting bodies and spores. The Barondes group has shown that the cells produce both surface lectins and receptors for them when cohesiveness develops. They think that the lectins, which are species-specific, enable slime mold cells of the same species to recognize one another and aggregate.

Gilbert Ashwell of the National Institute of Arthritis, Metabolism, and Digestive Diseases, and Anatol Morell of Albert Einstein College of Medicine have been studying the removal of glycoproteins from the blood by the liver of several mammalian species. (Most blood proteins, with the exception of albumin, contain carbohydrates and are thus glycoproteins.) The carbohydrate chains of the glycoproteins normally terminate with sialic acid.

The investigators observed that when the terminal sialic acid residues were removed to expose the penultimate residue, galactose, the glycoproteins were taken up very quickly and destroyed by the liver. They subsequently identified a liver receptor that specifically recognizes and combines with the galactose of the glycoproteins. On further analysis, the liver receptor turned out to be a protein with all the properties of a lectin. Ashwell and Morell have indentified another lectin-like protein in chicken livers that recognizes a different sugar.

Ashwell suggests that the concept of a lectin be broadened to embrace all biological systems, not just plants. He would define lectins as proteins with the capacity to recognize subtle differences in cell surface carbohydrate sequences as a means of regulating a variety of normal physiological functions.

Much of the evidence implicating lectins in recognition processes in plants is still circumstantial rather than definitive, and it is still too early to tell whether Ashwell's suggestion to broaden the definition of lectin will be accepted. Nevertheless, the current research is stimulating a new look at some familiar substances and, at the same time, providing a better understanding of several important biological processes, including the development of symbiotic relationships between legumes and nitrogen-fixing bacteria.—JEAN L. MARX

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