How Rhizobia and Legumes Get It Together

Formation of nitrogen-fixing nodules on legume roots requires an intricate interplay between the plant and the nodulating rhizobial bacterium

Within the past few years investigators have finally begun to understand the basis for the interaction between legume plants and their symbiotic partners—the rhizobial bacteria that live in nodules on the plant roots and convert molecular nitrogen from the air to ammonia, a nutrient essential for plant growth. In particular, the genes that are needed for formation of the nitrogen-fixing nodules are now being identified.

The work, much of which was described recently at the Sixth International Symposium on Nitrogen Fixation,* shows that several genes, both plant and bacterial, are required. Although a great deal still remains to be learned about these genes and their activities, the research shows that during nodulation there is an intricate interplay of events in which the plant influences the expression of the bacterial nodulation (*nod*) genes and the bacteria in turn influence the activity of the plant genes.

A few years ago, researchers learned that the rhizobial genes needed for nodulation are located on a large plasmid, which is called the symbiosis plasmid. It also carries the nitrogen fixation (*nif*) genes themselves.

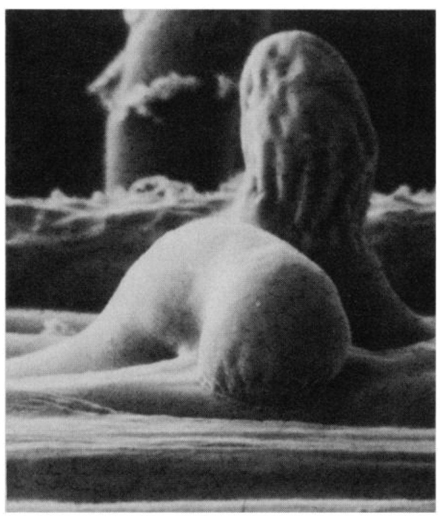
Among other things, this discovery challenged the previous definition of "species" for the rhizobial bacteria. A given bacterium can usually infect and nodulate a limited range of legume species, often just one, and the species of the bacteria have generally been defined by their host ranges. However, investigators soon found that many of the rhizobial bacteria could be induced to recognize a new legume partner simply by transferring into it a symbiosis plasmid from another bacterium with a different specificity. Introduction of a rhizobial symbiosis plasmid into *Agrobacterium tumefaciens* could even confer on this bacterium, which is better known as the cause of the tumorous plant growths called crown galls, the ability to produce root nodules, albeit defective ones that do not fix nitrogen.

But the main consequence of identifying the symbiosis plasmid was to allow researchers to begin identifying and isolating the rhizobial *nod* genes. At the symposium, data presented by representatives from four groups indicated that there is a consensus developing on the organization and expression of these genes.

*The symposium was held at Oregon State University in Corvallis on 4 to 10 August.

The groups contributing the data are those of Adam Kondorosi of the Biological Research Center of the Hungarian Academy of Sciences in Szeged, Barry Rolfe of the Australian National University in Canberra, Sharon Long of Stanford University, and Andrew Johnston of the John Innes Institute in Norwich, England.

The *nod* genes come in two categories.



A hair-curling experience

Legume root hair (foreground) curls to prepare for infection by rhizobial bacteria. (Source: Sandy Perkins and Harry Calvert of Battelle-Kettering Laboratory)

Four genes, usually designated simply as *nod* A, B, C, and D, encode proteins that have highly conserved amino acid sequences and perform common functions in the different rhizobial bacteria. In addition, there is another group of at least four genes that are apparently needed for recognition of specific legume hosts.

Analysis of the organization of the common *nod* genes revealed that the A, B, and C genes are located next to one another on the plasmid and are all oriented in the same direction. They are transcribed into messenger RNA as a unit. The D gene is next to the A gene, but is oriented in the opposite direction and is apparently transcribed separately.

Over the years, a variety of evidence has shown that the nodulation efficiencies of rhizobial bacteria are increased by exposure to plants or to plant exudates. These materials may be producing their effects by stimulating expression of the common *nod* genes, the new work suggests. The D gene is transcribed all the time in bacteria that are living independently from their legume hosts, whereas the A, B, and C genes are generally inactive under these conditions. The three genes are turned on, however, when the bacteria are exposed to material exuded by legume roots, provided that the D gene is already being expressed.

All legume root exudates produce this effect, which apparently does not contribute to the specificity of symbiosis, Long explains, but exudates from a variety of nonlegumes do not. The exudate component that works with the D gene product to activate the A, B, and C genes has not yet been isolated and characterized, but Long's work suggests that it has a low molecular weight.

Although very little is currently known about the exact functions of any of the *nod* genes, studies of mutants are providing some clues, especially to the activities of the common *nod* genes. When normal rhizobial bacteria interact with their legume hosts, one of the early structural changes in the plant root is a stimulation of the division of the cells in the cortical layer, which lies under the epidermal cells. This stimulation does not require that the bacteria attach to the plant roots, according to Wolfgang Bauer of Battelle-Kettering Laboratory in Yellow Springs, Ohio. It can be induced in soybean roots by a soluble substance that is released by the bacterium that nodulates this legume. The effect is specific in that other rhizobial bacteria do not stimulate cortical cell division in soybean roots.

The nodulating bacteria enter the roots of their symbiotic partners through the root hairs, epidermal cells that protrude by as much as a millimeter from the root surface. Before the bacteria enter, however, the root hairs curl. The cause of the curling is unknown, but it appears to be required for the bacteria to penetrate. The entering bacteria then form an infection thread by which they travel through the epidermal cells to the dividing cortical cells, which eventually give rise to the nodules.

Mutations in either the A, B, or C *nod* gene completely prevent nodule formation. Such mutants do not cause root-hair curling or form infection threads. Preliminary results from Long's laboratory indicate that the mutants also fail to elicit the early cortical cell divisions. Mutations that completely prevent D gene activity also prevent nodulation, although in *Rhizobium meliloti*, which has multiple D genes, a mutation in a single copy slows down the onset of nodulation but does not prevent it.

Work from Frank Dazzo's laboratory at Michigan State University suggests that some of the essential *nod* genes may participate in the synthesis of bacterial substances that are needed for interacting with legume roots. Dazzo and his colleagues had previously found that the specific recognition of clover roots by *R. trifolii* involves an interaction between a plant lectin, a glycoprotein that is found both on the root hairs and in clover root exudate, and polysaccharides on the bacterial surface. Dazzo hypothesizes that binding of the lectin, which is called trifoliin A, to the polysaccharide transmits to the bacteria a signal that triggers the responses necessary for successful infection of clover roots.

More recently, Dazzo's group, using mutant rhizobial strains prepared in Rolfe's laboratory, have found that mutations in the common *nod* genes A and D can not only prevent nodulation but may also decrease, by as much as 95 to 100 percent, the ability of *R. trifolii* bacteria to bind trifoliin A. Moreover, the investigators find the composition of the noncarbohydrate components of the lectin-binding polysaccharides to be altered in these mutants.

It seems somewhat surprising that mutations in the common *nod* genes could affect the synthesis of molecules involved in specific recognition. However, according to Dazzo, the evidence suggests that the genes do not code for the synthetic enzymes themselves but may produce products that help to regulate the highly complex pathways by which polysaccharides are synthesized. If that proves to be the case, then the products could have common functions in different rhizobial bacteria, even though the final products of the affected pathways might differ.

Not only can materials released by the plant affect expression of bacterial genes during the initiation of symbiosis, but materials released by the bacteria can also affect events in the legume root cells. The stimulation of the division of cortical cells is one example. Another was reported by Ton Bisseling of the Agricultural University in Wageningen in the Netherlands, who has been investigating the plant genes that are specifically expressed in the nodules. The activation of these genes follows a pattern in which one is turned on several days before the others. This early gene is a candidate to be a regulator of those that are expressed later.

According to Bisseling, the *nif* genes are not needed for the activation of the plant genes. The *nod* genes are, although they are not sufficient. Bisseling's experiments indicate that rhizobial chromosomal genes are also required to turn on the full complement of plant genes in the developing nodules.

All in all, the results described at the Nitrogen Fixation Symposium show that a great deal of progress has been made in the last year or two in identifying the genes that are needed to establish symbioses between rhizobial bacteria and their legume hosts. Although the picture is incomplete, it already shows that the interplay between the symbiotic partners is complex, requiring both to contribute to produce nitrogen-fixing nodules.

—Jean L. Marx

The Core of the Milky Way

Our galaxy appears to be a miniature quasar; all the evidence now points to an enormous black hole at the center

At the core of the Milky Way there lies a turbulent agglomeration of stars, gas, and dust, arrayed around a compact energy source that resembles nothing else in our galaxy. Recent findings, including some observations announced only this year, have made the conclusion almost inescapable: this energy source is a miniature quasar powered by the accretion of matter into a huge black hole.

Admittedly, our galaxy is a pretty dim candle as quasars go, by a factor of many thousands. In the accretion process, matter spiraling into the black hole is compressed and heated so much that it converts most of its mass into radiant energy before finally falling in. Real quasars are thought to be galaxies containing black holes of roughly a billion solar masses; presumably our own black hole is only a few million solar masses. But in any case the Milky Way is hardly unique: dim quasar-like emissions have recently been detected in the cores of many other normal-looking galaxies (*1*); moreover, intermediate-sized black holes also seem to be responsible for the very luminous nuclei of "active" galaxies, such as the Seyferts and the BL Lacertae objects. Thus, the nucleus of the Milky Way offers astronomers their best chance for a close-up study of a phenomenon that is ubiquitous in the universe.

Probably the best way to understand the galactic nucleus is to start by relating it to the Milky Way as a whole. Beginning from the outside and working in:

• The disk and the spiral arms. In terms of its spiral structure and virtually everything else, the Milky Way seems utterly typical. From our vantage point on the edge of one of the spiral arms, about 10,000 parsecs out from the center, the galactic nucleus lies in the direction of the constellation of Sagittarius. (A parsec is 3.26 light-years.) Unfortunately for optical astronomers, however, the disk of the galaxy is lined with masses of interstellar gas and dust that block every vestige of visible light from the nucleus. The central regions are thus the domain of radio and infrared astronomers, who work at wavelengths where the clouds are relatively translucent. By the same token, the process of understanding the nucleus has largely been a story of ever improving radio and infrared instrumentation.

• The central bulge. One of the most prominent features of the Milky Way, this slightly flattened sphere of stars sits in the middle of the spiral disk like the yolk of a fried egg. It is about 5000 parsecs in radius and is composed primarily of old, reddish stars, with rela-