NITROGEN FIXATION

RESEARCHERS PURSUE *NOD* GENES' TRIGGER

SWANSEA, U.K.—Discoveries are now beginning to link the genetics and biochemistry of nodulation of soybean, peanuts, and other legumes, Andy Johnston told the Annual Meeting of the British Association for the Advancement of Science. Johnston was speaking in a session on the environmental release of genetically engineered organisms, sponsored by the Institute for Scientific Information.

Recent work at several centers, including Johnston's laboratory at the University of East Anglia, Norwich, has begun to identify the substances that switch on the cluster of nodulation (*nod*) genes in rhizobia. These genes are responsible for the early stages of infection of legumes and also determine the particular host-range of specific rhizobial strains, making them targets for genetic manipulation as a means of altering and extending the host-range. When the bacteria are in the presence of a potential host legume, the *nod* genes are activated by phenolic compounds—flavenoids—exuded by plant roots. These are highly potent, requiring only a few thousand molecules per bacterial cell to turn on the genes at a high level to produce proteins that then trigger infection.

Nod genes have now been sequenced in several Rhizobium species, and the sequences compared with those in data banks. Although precise biochemical functions cannot yet be ascribed to individual *nod* genes, *nod*M encodes a protein similar to glucosamine synthase, *nod*L may be an acetylase, and the *nod*F and *nod*E genes are thought to be involved in the synthesis of a lipid moiety. "All of these deduced products are key components of one of the most exciting molecules of the decade—the tetra-acetylglucosamine identified recently in Toulouse, France, as the substance produced by *Rhizobium melitoti* that causes root hair curling in alfalfa."

The curling of root hairs is the first observable step in the infection process that leads to nodulation. It occurs when the *nod* genes, induced by exposure to the appropriate flavenoid made by the plant, produce a previously unknown hormone. Work by Patrice Lerouge and colleagues at the Centre de Recherches de Biologie Moleculaire des Relations Plantes-Microorganisms has now demonstrated that the tetra-acetylglucosamine derivative, which also contains lipid and sulphated substituents, is even more potent than the flavenoids that trigger the *nod* genes. It induces root hair curling at concentrations as low as 10^{-13} molar—making it millions of times more active than "classical" plant hormones such as auxins and cytokinins.

"The next, challenging stages in unraveling the story are to determine what receptor exists in the host legume which perceives this molecule, and then to work out how the resultant signal is transduced and results in the reprogramming of the pattern of plant development so that a nodule is formed," Johnston said. He believes that introduction of the root hair curling factor could provide a means of increasing the rate of nodulation in crops, such as soybean in North America, that are entirely dependent on the inoculation of soil with foreign strains of rhizobia.

—Bernard Dixon

ANIMAL VACCINE TRIAL

U.S. FIELD TEST UNDERWAY, ANOTHER PLANNED

WASHINGTON, D.C.—Fighting late-summer heat, humidity, and swarms of insects, a research team coordinated by the Wistar Institute (Philadelphia, PA) recently distributed 3,000 rabies-vaccine-laden baits in rugged upland regions of Parramore Island, Virginia. The coastal island thus became the site for the first U.S. field test of a genetically engineered vaccine. The test, provisionally approved in early 1989 by the U.S. Department of Agriculture (USDA), finally passed muster with officials from the Nature Conservancy (NC, Arlington, VA), which owns the island.

"I'd classify the test as a success at this point," says NC's Barry Truitt, who has for several years been tracking plans for the field test. So far, the baits were avidly devoured during the 10 days they were made available to island animals, he adds, with no adverse effects on island wildlife noted. Indeed, according to Truitt, the only known adverse effects occurred among the couple of dozen researchers who monitored the test. "Two of them had to go to the emergency room because of chigger bites," he says. "It's not a pleasant island to work on, particularly in August....My hat's off to them." Analysis of how well the vaccine works under field conditions—whether raccoons that consumed it develop antibody levels adequate to protect them against rabies virus—is still at an early stage. Monitoring of raccoons and other wildlife on the island will continue for a full year.

Before beginning the test, NC officials insisted that Wistar meet three major conditions, Truitt says. Thus Wistar agreed to provide NC with full insurance coverage, indemnification, and a major say in designing the field trial and animal monitoring protocols. In meeting the last requirement, Wistar also agreed to work with biologist Ray Dueser, a specialist in the ecology of small mammals, who has been studying wildlife on Parramore Island with National Science Foundation support.

"It took quite a while to agree, and working out the details was a long, drawn-out process," says Wistar spokesman Warren Cheston. The most difficulty arose in satisfying NC's desire to be indemnified against any lawsuits that might arise because of the field test. In making these arrangements, Wistar turned to its European corporate partner on the vaccine development project, Rhone-Merieux (Lyon, France), for backing.

Although testing the engineered rabies vaccine has been dogged by controversy, the risks involved are considered "extremely remote—the biology of rare events," according to Charles Ruprecht of Wistar, who has led the institute's efforts to develop and test the vaccine. In collaboration with scientists at Rhone-Merieux, Wistar researchers based the anti-rabies vaccine on vaccinia virus, which was used widely in human medicine to combat and, eventually, to eradicate smallpox. The engineered version of vaccinia (VRG, for vaccinia rabies glycoprotein) carries the gene for a key glycoprotein found on the surface of the rabies virus. When treated with VRG in laboratory tests, animals of several species develop immunity to rabies, according to Ruprecht. A similar vaccine is being tested extensively in field trials in Europe.

With the first U.S. field test of VRG under its belt, the Wistar team now is planning a mainland test for a site in northeastern Pennsylvania.

—Jeffrey L. Fox