MEETING REPORT

HOW SOON FOR NITROGEN FIXING PLANTS?

CORVALLIS, Ore.—Improvement of nitrogen fixation in plants has been one of the most discussed long-term goals of genetic engineers. And with good reason. The world's population may double in the next century, yet the global food supply is limited by fixed nitrogen. How far off does a product still lie? To judge from the Sixth International Symposium on Nitrogen Fixation, held at Oregon State University August 4–10, the short answer may be "closer than it used to."

The first products of the new engineering will no doubt be improved symbiotic rhizobia that fix more nitrogen for their host legumes. More ambitiously, scientists might broaden rhizobial host range, or augment the effects of root-associated azospirilla, to yield efficient fixation in plants such as cereal grains. The Holy Grail, of course, is the nitrogen-fixing corn plant made by direct insertion of cloned bacterial *nif* (nitrogen fixation) genes. Such practical matters, however, will have to await clarification of the fundamental aspects that were the province of this symposium.

At the leading edge right now is the genetics of rhizobial nodulation, as described by a number of groups from around the world. The most dramatic results concern the communication between plant and rhizobium that obviously goes on but has yet to be deciphered. Although the mechanics of nodulation are still obscure, several groups have begun localizing at least some of the *nod* (nodulation) gene products to the bacterial surface, where logic would have them, and are studying regulation of *nod* genes by inserting a reporter gene for *Escherichia coli* betagalactosidase. As shown most extensively for alfalfa rhizobium by Sharon Long's group at Stanford University, a small (~3,000 D) molecule exuded by roots (and seeds) can, together with the constitutive *nodD* gene product, induce expression of the *nodC* gene.

Communication may also somehow involve rhizobial surface polysaccharides, in which there has been a surge of interest. These may interact with plant lectins, for whose involvement there is at last genetic evidence. This includes mutant studies with clover rhizobium by Frank Dazzo (Michigan State University, East Lansing), and suppression by purified lectin of a soybean rhizobium mutant by Gary Stacey (University of Tennessee).

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REASONS

Rhizobia in the early stages of infection. Photo depicts an infection thread (running from upper left to lower right) containing nitrogen-fixing bacteria that are transversing and infecting cells of an alfalfa root nodule.

Current progress is mostly with the fast-growing rhizobia (associated with alfalfa, clover, pea, and bean) though that for the slow growers (with soybean and tropical legumes) is catching up. This has revealed some differences but many similarities among the various rhizobia. (Work is also beginning on frankia, the unrelated actinomycete that nodulates mainly forest trees.)

Characterization of rhizobial *nod* genes is ranging from analysis of DNA sequences to a growing correlation of genes with morphologically defined plant responses. In addition, *Fix* (fixation) genes controlling bacterial differentiation are starting to be identified, and nitrogen and carbon metabolism are being addressed Also, researchers are studying the uptake hydrogenase activity that in some rhizobia siphons energy from nitrogenase. It is thus ripe for engineering away.

Fixation by direct *nif* transfer will require, quite apart from vectors, well-defined genes and enzymes. Progress is still quite slow in establishing the physiological roles of the 17 known *nif* genes in the best-studied bacterium (*Klebsiella pneumoniae*). That work was summarized at the meeting by Frank Cannon of BioTechnica International (Cambridge, MA). Gains are likewise slow in unraveling the chemistry of the nitrogenase activite site, as well as in the details of stoichiometry, oxygen sensitivity, and ATP requirement. But regulation of gene expression is moving much faster, thanks to several groups, including S. C. Shen's (Shanghai Institute of Plant Physiology, People's Republic of China). Consensus promoter sequences have lately emerged both for genes activated by the *nif*-specific *nifA* product (evidently an RNA polymerase sigma factor), and for those activated by the general metabolic *ntrA/ntrC* products.

Nif genes are turning out to be similar in a variety of other bacteria, too. Data thus far are mainly for nitrogenase structural (*nifHDK*) genes, long known to be conserved in evolution, and, more recently, for *nifA* and *ntrA/ntrC* regulators (Fred Ausubel's group at Massachusetts General Hospital, Boston). How comparable other *nif* funtions are remains to be seen. There have already been surprises, however, such as the reiteration in rhizobia of *nif* and *nod* gene sequences, and the excision in cyanobacteria of a sizable (11 kb) chunk of DNA from within the nitrogenase *nifD* gene during heterocyst differentiation. The significance of neither process is yet understood.

Several groups are checking nodule-specific plant proteins, including the oxygen carrier leghemoglobin. Other groups are trying to select legume lines with improved fixation. The question is how soon breeders can profit from rhizobial advances. A major problem, still unsolved, is that in the field laboratory-modified rhizobial inoculants are invariably outcompeted by indigenous strains.

Altogether, the sense at this symposium was of basic research on nitrogen fixation begining to come into its own. If other fields are any guide, application may not be far behind.