

## 'Nif' and 'hup' genes

Raymond C. Valentine

All crops need nitrogen for growth. Nitrogen fixation—the enzymatic conversion of atmospheric nitrogen gas into a form available to the plant—is a trait that only some bacteria have evolved.

If plants such as soybean form a symbiotic association with nitrogen-fixing bacteria, nodules develop on their roots, and the plants can become independent of soil or chemical nitrogenous fertilizers. Obviously, improving the efficiency of symbiotic nitrogen fixation by leguminous crops and genetically engineering new crop plants that produce their own nitrogen supply would be of tremendous benefit to agriculture. Other University of California scientists at Davis are devising strategies to improve nitrogen fixation efficiency. My primary goal is to use genetic engineering techniques to isolate and study the genes responsible for nitrogen fixation.

The *nif* (nitrogen-fixation) genes were isolated from an important variety of soybean root nodule bacterium, *Rhizobium japonicum* strain 110. After DNA from this *Rhizobium* species was purified, random fragments were inserted into a plasmid vector, which was used to transform *Escherichia coli*. Calculations showed that the total chromosome of *Rhizobium* should be represented in about 3,000 to 4,000 independent *E. coli* transfor-

mants. Among the 3,325 *E. coli* transformants screened, 21 contained a piece of DNA with the *nif* genes. Analysis of the plasmids in these 21 strains showed that all had the same size piece of DNA of  $5.8 \times 10^6$  daltons. Further experiments confirmed that this piece of DNA carried the *nif* genes.

The availability of cloned nitrogen fixation genes from *Rhizobium japonicum* now makes it possible to study their organization and mode of expression. Ultimately the *nif* genes may be used for genetic engineering of new crop plants, but this is long-range research with many hurdles yet to be overcome.

A lot of energy is wasted during nitrogen fixation through the loss of the hydrogen gas released by many nodules. One researcher calculated that the energy loss in the form of hydrogen from soybeans in the United States alone was equivalent to the total supply of energy used in England over a three-month period.

Some strains of *Rhizobium japonicum* turn out to have genes for hydrogen uptake (*hup* genes), which means that soybean plants whose root nodules contain these strains are more energy-efficient than the other plants. Scientists in my laboratory have recently discovered that the *hup* genes are situated on plasmids, which can be transferred from one bacterial cell to another by conjugation. This discovery raises the possibility of introducing these plasmids, or smaller derivatives of them, into *R. japonicum* strains that lack them, thus increasing energy efficiency without significantly reducing the strain's ability to compete in its original environment. This is important, because local soil and other environmental factors such as pH, drought, salt, toxic ions, and so forth may largely prohibit the introduction of new rhizobial strains where local varieties

have evolved to be far more competitive.

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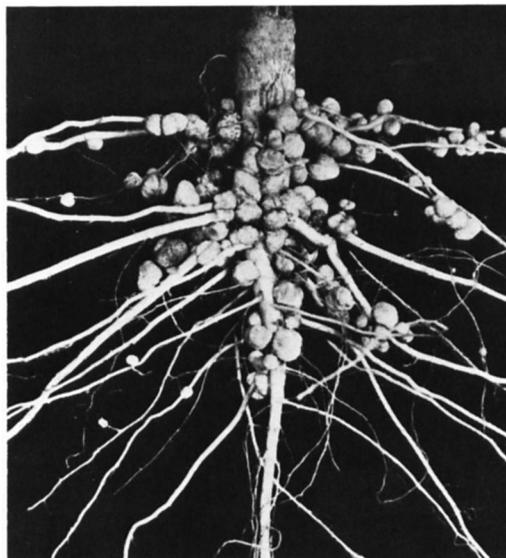
## Maize anaerobic genes

Michael Freeling

A higher plant has between one and ten million genes, each of which holds information that specifies the structure of a product RNA and, often, a protein. In addition, each has nucleotide sequences involved in recognizing on-off signals. Using recombinant DNA technology, pieces of DNA carrying one or a few genes can now be removed from practically any organism, replicated in microorganisms, and then studied at the level of nucleotide sequence and sequence arrangement. A few genes from higher plants are now being analyzed at this level, and alcohol dehydrogenase-1 (*Adhl*) in maize, the gene studied most intensively in my laboratory, is one of them.

A gene serves the organism not only by encoding a product, but also as an integral part of the organism as a whole. For example, the alcohol dehydrogenase enzyme is formed in some organs and cell types of maize but not in others. In some organs *Adhl* is expressed naturally, but in others it is expressed only under anaerobic conditions. The *Adhl* gene is woven into a complicated net of gene circuits that respond both to internal, developmental cues and to external, environmental stimuli. To work backwards from specific genes to the now unknown levels of gene regulation is my research focus.

*Adhl* enzyme activity is absolutely required if a maize seed or seedling is to survive drowning for more than a few hours. When maize seedlings are subjected to an anaerobic environment, most protein synthesis halts. After a few hours, *Adhl* and about nine major and ten minor proteins are synthesized. To prove that some or all the anaerobic proteins cause flood tolerance is extremely difficult. The only approach that might suffice, and the one we are using, is to obtain maize mutants or cultivars that are flood-tolerant and to find out how many and which genes are involved. We have been particularly successful in isolating mutants of *Adhl*. Some of our mutants alter the stability of enzyme expression, others the quantity. Some are restricted in their organ specificity, others show



Soybean root nodules—miniature nitrogen-manufacturing factories—provide the plant with fixed nitrogen from atmospheric  $N_2$  gas. Some nitrogen-fixing bacteria also have genes for hydrogen uptake, recycling  $H_2$  produced by the reaction. Examples of important nodulated crops in California are alfalfa, field beans, and clovers.