After taking his B.Sc. and M.Sc. in plant morphology at the University of Agra he left India for Canada, and in 1970, finished his Ph.D. at the University of Western Ontario, where he worked on the ability of plants to survive ionizing radiation. For two years he studied at the Institute of Cancer Research in Philadelphia — "a very active, competitive place," he says — and then on to McGill, where he was attracted to a field just coming of age — plant molecular biology.

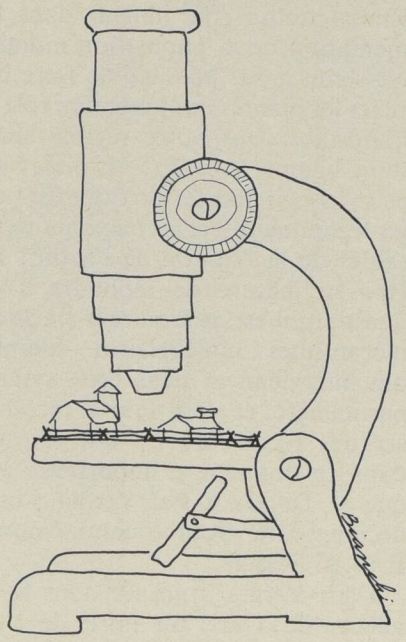
Early in the 1970's many molecular biologists were enthusiastically talking about teaching plants to fertilize themselves by providing them with nitrogen-fixing genes from bacteria. To Verma, these plans sounded more difficult than breeding apples with oranges. "I became somewhat dubious," he says. "Even if you were physically successful in putting bacterial genes directly into plants you then have to get the plant to express them — in other words, to synthesize the enzyme nitrogenase that fixes nitrogen, and to support this process with the biological energy it needs. The problem here is that while the plant cell needs oxygen to live, nitrogenase is very sensitive to oxygen. The strategy I developed was to leave the nitrogen-fixing functions where they are, in the bacteria, and try to understand the leguminous plant genes that are involved in the symbiotic relationship; then, once we learned to manipulate them, transfer the ability to cooperate with bacteria from legumes to other plants." Easily said, perhaps, but much more difficult to carry out.

"Searching for all the genes involved in symbiosis," says Verma, "is like searching for a black cat in a dark room — there are very few landmarks to guide you."

In a series of elegant and subtle experiments, Verma and his collaborators have pinned down, at least in outline, their elusive quarry.

"I began looking for a model system that would allow me to study a specific plant gene," he explains. "Usually, a plant cell does not make abundant quantities of any gene product, but legume root nodules right away caught my attention; they contain an abundant protein known as leghemoglobin."

Found only in legume root nodules, leghemoglobin is remarkably similar, in color and function, to the hemoglobin which colors human blood red, and which carries oxygen from our



lungs to our cells. If you slice open a soybean nodule with your fingernail, you see that its interior is tinted pink by leghemoglobin. Like our hemoglobin, the plant molecule is composed of a protein component and a so-called heme ring which binds the oxygen.

What Dr. Verma wanted to do was isolate the gene in the plant cell that held the code for making leghemoglobin, not in the form that it is found in the nucleus, as DNA, but as it is expressed at the site where protein synthesis takes place, as messenger-RNA. Many people were trying to isolate such sequences from eukaryotic cells (cells with a nucleus) but few had succeeded.

Genes are short segments of a twisted, double-stranded molecule known as DNA. Like letters spelling out code words, the chemical units strung out along each of its strands carry instructions telling the cell exactly what to do. The message is copied — and somewhat edited — to form a single-stranded molecule, messenger-RNA, the mirror image of a DNA strand. Messenger RNA carries the genetic instructions out from the nucleus to the cell's workbench where they are translated into the assembly of amino acids that make up proteins like leghemoglobin. DNA makes RNA; RNA makes proteins; proteins make us and all other living things.

What Verma did was separate the messenger RNA sequences found outside the nucleus of an infected soybean root cell by their size, using techniques called ultracentrifugation and electrophoresis. Since leghemoglobin is the most abundant protein in these cells, he reasoned that it must be coded for by the messenger-RNA which occurred most frequently. To clinch his identification, he mixed this abundant messenger-RNA with a laboratory system that translates the messenger-RNA code into protein; the system obediently manufactured leghemoglobin.

The next step, putting his hands on the actual gene for leghemoglobin, the DNA from the soybean cell nucleus, was not so easy — nor, for that matter, was it cheap. It required the expansion of Verma's "molecular farm" and the purchase of elaborate equipment — including a lab bench covered by a negative-pressure hood for containing cloning biohazards — and expensive enzymes, isotopes and other supplies. It required the cooperation of students, fellow scientists in a number of countries, the application of the latest techniques of molecular biology, and many long hours of work.

Here, in brief, is how it was done. The entire set of soybean genes, its genome, was snipped up into millions of short fragments of DNA. Each fragment was then inserted into a bacterium and cloned, thereby creating a huge gene "library". The problem now was to fish out from a labful of test tubes the clone containing the DNA fragment coding for leghemoglobin. Verma's solution was to make a probe, a radioactively-labelled molecule similar in structure to leghemoglobin DNA, and hence with a tendency to bind to it.

David Thomas, a molecular geneticist at the National Research Council in Ottawa, helped construct the probe. In Montreal, rough versions of the desired DNA were made, modelled on the RNA which Verma had already isolated, and shipped to Thomas in Ottawa, who then inserted these gene copies into bacteria, cloned them, and sent them back. Verma then isolated the cloned DNA, tagged it with radioactive tracers, and used it to screen the gene library of the soybean cell. "It was a very complex system," he says. "And it took years of hard work." The first soybean genomic libraries screened with this "hot" probe failed to yield a leghemoglobin gene, but finally not one but two clones "lit up". In the February 5, 1981, issue of *Nature*, Verma and his co-workers re-