A gene at last

After six years of work, Nobelist Khorana has synthesized DNA

At a small biochemistry colloquium this week at the University of Wisconsin, Dr. Har Gobind Khorana reported that he had made a gene. His colleagues at the Madison campus and elsewhere say it is typical of the Indian Nobel Laureate to make such a significant announcement in such a modest forum. It marks the first time scientists have created an entirely artificial, complete gene, and is an achievement Dr. Khorana has been striving for during most of his professional life.

In 1964, Dr. Khorana and his associates were the first to produce synthetic polynucleotides, bits of DNA (deoxyribonucleic acid) that they hoped eventually to link together into a total DNA molecule. In the six years since that first triumph, which contributed to his Nobel Prize, Dr. Khorana and his team of scientists from six nations have been refining and expanding their chemical techniques.

The work led to the synthesis of the gene, a short molecule of DNA, that codes for production of alanine transfer-RNA in yeast.

This particular t-RNA, which plays an essential and well defined role in natural protein synthesis, was selected as a model from which to work, Dr. Khorana says, because at the time he began his experiments, it was the only t-RNA of known structure. The structure had been determined previously by Dr. Robert Holley, now of the Salk Institute, who shared the 1968 Nobel prize with Dr. Khorana and Dr. Marshall Nirenberg of the National Institutes of Health (SN: 10/26/68, p. 411).

Knowing the order of the nucleotides in the t-RNA molecule, Dr. Khorana deduced the probable order of nucleotides in the DNA molecule that codes for its production. Then, using commercially available nucleotides, the four bases or components of DNA—adenine, thymine, guanine and cytosine—he linked them to molecules of sugar and phosphoric acid in the same order in which they occur in the natural yeast gene.

His technique is similar to that for synthesizing a protein, a long chain of amino acid molecules. The gene synthesis involves first the production of small polynucleotides and then their linkage into the entire DNA helix, 77 nucleotides long. According to Dr. Khorana, it is better to make a large number of short pieces than to try to make one single strand, nucleotide by nucleotide, a process in which errors might easily accumulate.

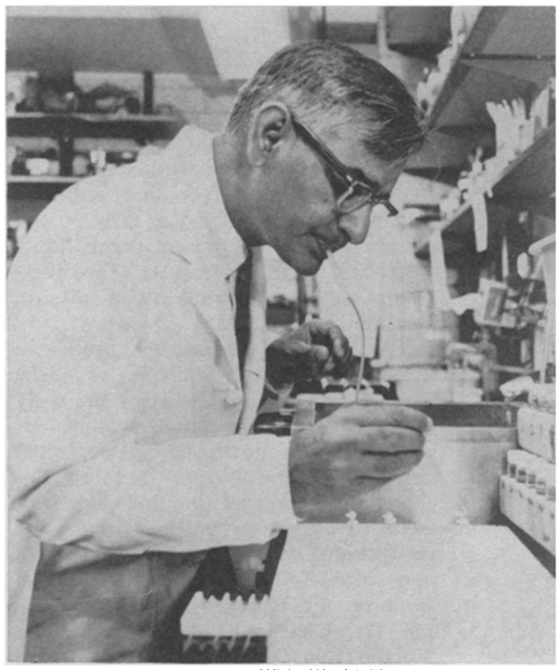
Initial confirmation of the fact that the laboratory gene was indeed identical to its natural counterpart came from chemical analysis.

Ultimate proof will come, Dr. Khorana says, when he is able to copy the synthetic gene by using DNA polymerase, an enzyme key to DNA replication. Next he will attempt to produce t-RNA from his artificial gene. Then he would like to test the gene's activity in a living cell. For this, however, he will need a mutant yeast cell that lacks alanine t-RNA. If one is found, he will use a virus carrier to introduce the synthetic gene into the mutant cell. If it is active, it will code for t-RNA.

In the interim, Dr. Khorana is working on the synthesis of a second gene, one that codes for tyrosine-suppressor t-RNA in the common bacteria *E. coli.* Because a mutant *E. coli* deficient in this specific type of t-RNA is already at hand, it will not be difficult to test the new gene in this living system. At present, most of the fragments for the second gene have been made. Dr. Khorana expects to have them joined into a whole molecule within a few months.

Although Dr. Khorana has been striving for gene synthesis for several years, the job was all but completed a few months ago. Assessing the significance of the present achievement, one colleague comments, "The intellectual framework for gene synthesis, provided in large part by Dr. Khorana himself, has been known now for some time. With the actual creation of an artificial gene, nothing really new has been learned; but it is a symbol of great progress."

The achievement does open the door to the manufacture of other genes and gives scientists a new route to follow in their attempts to understand the chemical processes of life. "Most importantly," says Dr. David Green, co-director with Dr. Khorana of Wisconsin's Institute for Enzyme Research, "it means that investigators will be able to modify gene structure and then test the consequences of that modification by introducing these new and specialized DNA molecules into cells.



Wide World Photos

Dr. Khorana: first gene synthesis.

Dr. Khorana agrees. "It is clear," he has said, "that eventually our ability to manipulate the information content of DNA, which we equate with the sequence of its nucleotides, is dependent on our ability to put together the information by chemical synthesis." Previous success in creating genetic material in the laboratory, most notably Dr. Sol Spiegelman's work with RNA (SN: 10/9/65, p. 227) and Dr. Arthur Kornberg's synthesis of a piece of viral DNA (SN: 12/30/67, p. 629) was accomplished with the aid of bits of natural genetic material as a model for replication rather than total chemical synthesis of the kind Dr. Khorana achieved.

At first, gene mutation will focus on small yeast, bacterial and viral DNA's. Synthesis of a human gene, millions of nucleotides long, is still years away. □

LEG 11

The old and the mobile

Glomar Challenger, continuing its unprecedented odyssey into the oceans' past, set out on its eleventh voyage two months ago from Miami. This time it was after the first sediments laid down after the birth of the Atlantic Ocean. This week it docked at Hoboken, N.J., its mission accomplished. Aboard the scientific drilling vessel were cores from the western North Atlantic containing the oldest material ever obtained from an ocean floor: sediments laid down 150 million to 160 million years ago.

Sediments 140 million years old had been obtained during previous legs of the project in the Western Atlantic and