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.

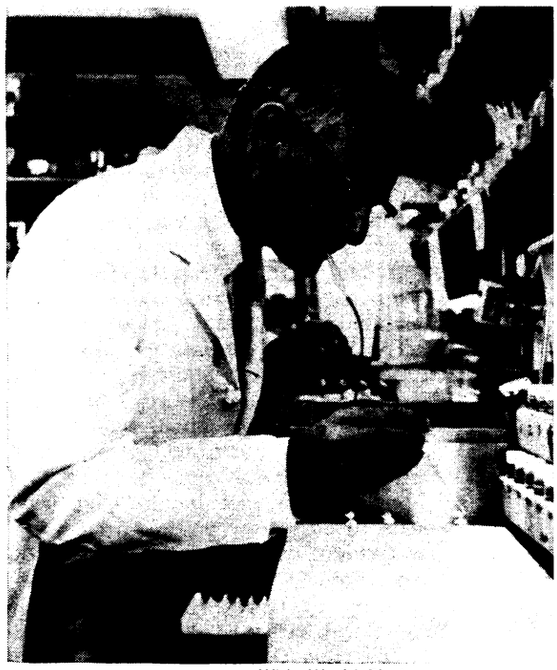
Initial confirmation of the fact that the laboratory gene was indeed identical to its natural counterpart came from chemical analysis.

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

the Northwestern Pacific. But in those cases the bit had not been able to penetrate to the lowest—and therefore oldest—deposits. The work emphasizes again the curious fact that although oceans have existed since early in the earth's history, the present ocean floors are relatively young features. The oldest continental rocks found, in contrast, are 3.5 billion years old.

The Jurassic limestones found in Leg 11, deposited directly over the basaltic bedrock floor, portray the early history of a young, small, shallow Atlantic Ocean; limestone is deposited only in shallow water. "I think this agrees with the fact that we have sediments deposited when the ocean was very young, before it got very deep," says John I. Ewing of the Lamont-Doherty Geological Observatory. He and Dr. Charles D. Hollister of the Woods Hole Oceanographic Institution were co-chief scientists for Leg 11 of the Deep Sea Drilling Project, sponsored by the National Science Foundation and managed by the Scripps Institution of Oceanography.

Their data indicate that the Atlantic Ocean floor is widening at an average rate of three centimeters a year. At this rate the ocean would have reached its present dimensions in about 175 million years. The work is thus strong supporting evidence that Europe and North America broke apart no earlier than about 180 million years ago.

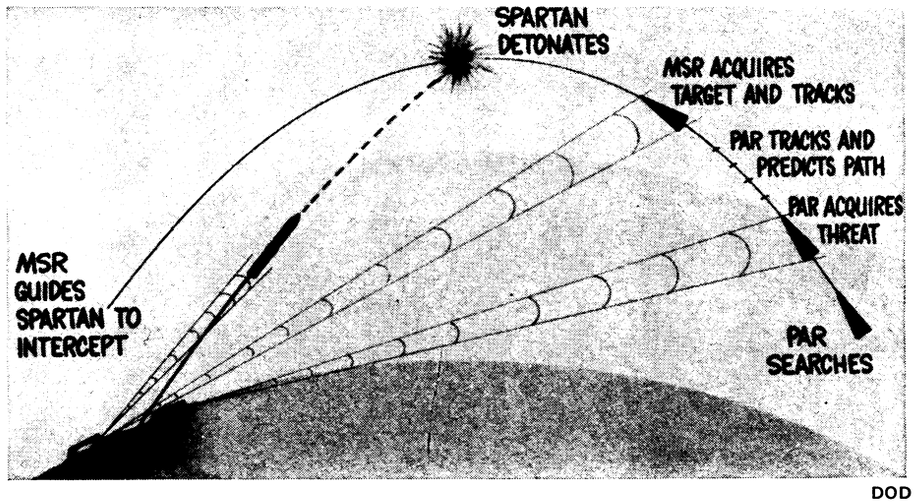
The scientists on Leg 11 also drilled off the coast of Florida into a massive accumulation of land-derived sediments, comparable in volume to the Appalachian Mountains. They found it contains a considerable amount of spruce pollen. Spruce has never grown in the southeastern United States. This and the sediments' mineral composition suggest that the sediments were originally eroded into the ocean from the northeastern part of the continent and transported south by strong bottom currents. This further dispels—at least for the extreme western North Atlantic—a once-prevalent textbook view that the ocean floors are quiet places displaying little erosion or current motion.

At one site during Leg 11, some 300 miles east of Cape Hatteras in sediments associated with the continental margin, the scientists drilled to a record depth of 3,320 feet below the ocean floor. The earlier record was 3,231 feet, in the Pacific Ocean.

Later this week the Glomar Challenger was to sail 300 miles offshore from New York for two weeks of tests of a system to enable a drill stem to be withdrawn from a hole, fitted with a new bit and then replaced in the same hole. The system has never been tested in deep water, but project managers were hopeful for success. □

SAFEGUARD DEBATE

Shifting the ground



DOD

Safeguard's first line of defense depends on early radar detection.

In its long road from the Nike-Zeus of the 1950's through President Johnson's Sentinel plan for protecting United States cities from missile attack to its present form, the Safeguard Antiballistic Missile system has collected a number of reasons for being: It is designed to protect against a direct Soviet attack on the Minuteman offensive missiles, against a primitive, less sharply focused Chinese attack in this decade, or an accidental attack.

Last year the Nixon Administration won a fierce battle over deployment of the first phase of the Safeguard system. This year a proposal for a second phase passed the House by a 326-69 vote and is now before the Senate's Armed Services subcommittee; both critics and proponents are girding for another Senate showdown in July.

The entire $10.7 billion program will this year require under $100 million for one Phase II site in addition to completion of two Phase I sites. It has been repeatedly bombarded with ethical, economic and technical attacks (SN: 8/16, p. 127), as well as the contention by scientists and engineers that the system will not work, or do what the Administration claims it will do.

Any missile defense system is by definition complex. Safeguard combines a multitude of sophisticated subsystems that must function as an integral unit; its difficulties are compounded by the need to deal as well with offensive measures designed to confound it. Within less than 30 minutes, the radar-computer-missile system must spot a hostile missile, compute its trajectory, calculate an intercept point, fire a long-range Spartan missile to intercept, alert short-range, high-speed Sprint missiles of incoming warheads missed by Spartan, discriminate between warheads and decoys and fire the Sprint.

DOD

Sprint intercepts below 100,000 feet.

Crucial to the system is the radar. Missiles can be detected at launch but their trajectory cannot be determined. That is the job of the long-range Perimeter Acquisition Radar (PAR), which can look at many objects at once and picks up hostile missiles as soon as they show up over the horizon. The Missile Site Radar (MSR) takes over from the PAR at closer range.

Most scientists and engineers would admit that this system, given enough time and money, would not be impossible to build.

At one point in last year's debate a criticism was that the radars, in exposed installations, were vulnerable to attack. This was met by a subsequent announcement by Defense research chief Dr. John S. Foster that funds were being sought to develop smaller