First Total Synthesis of a Mammalian Gene

It's hard to believe that in a swift quarter-century, biologists have made the quantum leap from the identification of hereditary material to its synthesis. Yet that is precisely what has happened, thanks to the audacity of biologists and the ever-increasing array of tools at their disposal.

In 1970, a gene was synthesized for the first time, by Nobel laureate Har Gobind Khorana and his team at the Massachusetts Institute of Technology. It was a yeast gene (SN: 6/6/70, p. 547). Subsequently they synthesized a bacterial gene that had the potential to function detectably within the living cell (SN: 9/1/73, p. 132). Then in 1972, a mammalian gene was partially synthesized for the first time, by three independent research groups at MIT, the National Institutes of Health and Columbia University. This was the rabbit gene that makes hemoglobin (SN: 2/5/72, p. 86).

Now the rabbit hemoglobin gene has been totally synthesized by Harvard University biologists, marking the first total synthesis of a mammalian gene. The coup also opens the door to learning more about gene action and regulation, the origin of sickle cell anemia and other genetic diseases and even to ultimately correcting these diseases by replacing the faulty gene with a synthetic, healthy one. The investigators are Argiris Efstratiadis, Fotis Kafatos, Allan Maxam and Thomas Maniatis. Their results will be published in the February CELL.

Efstratiadis and his colleagues first purified a strand of rabbit messenger RNA—the nucleic acid that transfers the genetic instructions in a cell into a protein, in this case hemoglobin. Then they took the so-called reverse transcriptase enzyme, which is able to convert mRNA molecules into DNA molecules (genes). Such a conversion is the reverse of the usual DNA-into-RNA process that occurs in cells. They learned how to use the reverse transcriptase enzyme to make a full-length DNA copy of rabbit hemoglobin mRNA.

Those investigators who had previously tried to make such a copy with the reverse transcriptase had always gotten partial products—tiny pieces of DNA. The reason that Efstratiadis and his co-workers succeeded is that they used a large enough concentration of nucleotides (precursor DNA material) to make the DNA copy.

During these experiments, the Harvard biologists also noticed that a tiny amount of DNA that they made was resistant to an enzyme called a nuclease. On the basis of that discovery and other investigators' findings, they suspected that there was a hairpin curve at the end of their DNA copy. If this was indeed true, they reasoned, then it should be possible to extend that hairpin to make a second complementary strand of the DNA copy. They attempted to do just that, using an enzyme known as DNA polymerase, and succeeded. Then they used a third enzyme, a nuclease, to snip off the two strands at the spot marked by the loop. The two strands thus constituted the rabbit hemoglobin gene.

This gene contains 650 nucleotides. It's as long as a human gene. The bacterial gene that Khorana and his team synthesized was only 80 nucleotides. Khorana and his colleagues also used a different method to synthesize their genes. First they deciphered the nucleotide sequence of those genes. Then they made synthetic copies of the genes by linking nucleotides together in the right order.

The importance of the Harvard investigators' technique, Maniatis explained to SCIENCE NEWS, is "that you can use the approach to make a double-stranded DNA molecule from any mRNA molecule you can purify. The number of genes you can isolate is simply proportional to the amount of mRNA starting material."

This technique could be used to explore how healthy genes work. For instance, the gene of interest could be synthesized, then transferred to rapidly multiplying bacteria in order to make large amounts of the gene. (Such a gene transfer technique is already possible; it does not fall into those categories of gene transfer that biologists are now restricting for safety and ethical reasons. See next article.)

The technique also offers approaches to determining how diseased genes operate. For instance, the gene that makes the abnormal hemoglobin responsible for sickle cell anemia might be synthesized and then mass-produced, in order to examine exactly why it causes disease.

Finally, the technique may help lead to the no-longer-science-fiction possibility that people might receive synthetic healthy genes to replace their diseased ones.

Although the Harvard team plans to synthesize more genes in the future, they are now concentrating on using their gene synthesis technique to learn more about gene regulation during animal development. Specifically, they have learned how to synthesize a full-length DNA copy from mRNA's in the silk moth. The silk moth, Maniatis explains, "is an ideal system for studying development biology, because the number of mRNA's is produced in sequence during development. What we hope to do is to isolate and clone each one of these and use those as a probe to study the regulation of genes during this development." □

Rules created to control DNA research

Janet L. Hopson

Twenty scientists squeeze three guideline versions in two days into one set of rules.

After three false starts, a group of biologists has managed one of the hardest translations of the year—changing the general edicts of the now famous Asilomar gene conference into specific ground rules for controlling the new technology of recombinant genetic engineering. These ground rules had to read out in terms of both development and safety. They had to allow the promise of genetic manipulation for medicine, agriculture and industry, but prevent the potential dangers of releasing uncontrollable recombinant organisms into the environment. Guideline writing, it was clear from the meeting, has its pitfalls, too.

The biologists—14 voting committee members, 3 administrators and a handful of consultants—constitute the National Institutes of Health advisory committee for recombinant DNA research, a program funded, in large part, by that agency. The committee was first formed in February after the Asilomar conference (SN: