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:

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3/8/75, p. 148) and has wrestled all year with the details of self regulation only sketched in by the larger, international group. The advisory committee, headed by NIH administrators DeWitt Stetten and Leon Jacobs, met last Thursday and Friday in La Jolla, Calif.

The difficult job of translating general guidelines into specific ones was complicated by growing dissension, both inside and outside the committee, over just how that translation should read. The committee came to La Jolla with three separate versions of proposed guidelines, written since February, and by some minor miracle, arrived upon an acceptable compromise within the two-day meeting. One set was drafted at Stanford this spring. Another, which substantially weakened the first, was drafted at a July meeting at Woods Hole, Mass. The third set was written this fall following an outcry from scientists—including Asilomar organizer Paul Berg of Stanford—concerned by the weakened controls over potentially dangerous experiments.

Distilling the results enormously, the full committee at La Jolla decided as follows: The containment of DNA recombinant experiments will be accomplished by both physical and biological means. Physical levels are classified P1, P2, P3, and P4, and range from no special equipment or microbiological techniques to the use of air locks, negative air pressure and decontamination of all laboratory waste materials. Biological containment will fall into three levels: EK1, the use of standard *E. coli* K-12 organisms and virus or plasmid vectors (messengers for carrying new genetic information into host cells); EK2, hosts and vectors that have been genetically altered to reduce their ability to survive outside the laboratory; EK3, EK2-level organisms that have been tested and shown not to survive in nature.

The most significant part of the new guidelines is the assignment of types of experiments to levels of containment. These assignments depended on the committee's assessment of the dangers to man and nature involved in the "worst possible scenario" for each type. Experiments involving DNA from primates must have high containment, P4 and EK3 or P3 and EK3. Containment levels decrease as one moves down the phylogenetic list from mammals through birds, cold-blooded vertebrates, invertebrates and lower eukaryotes. Similarly, higher plants require more stringent containment than lower plants. When pathogenic hosts or vectors are used, containment levels, are, in general, increased. When sterile embryonic tissues or purified DNA fragments are used, containment levels can be decreased.

In every case, the La Jolla levels match or exceed those suggested at Asilomar. A few experiments done since February have convinced many researchers that the chance of a dangerous accident is much smaller than presumed by those who initiated the present self-regulatory measures last year (SN: 7/27/74, p. 52). But there are still gaps in understanding both potential dangers and benefits that are, in one committee member's words, "big enough to drive a truck through."

Scientists, both on the committee and in the research community at large, are divided into two groups (and the shades in between): liberals who argue that scientific progress will be impaired by overly strict guidelines, and conservatives who feel that containment should be high, even if it slows research. The conservative position won out at La Jolla, for the most part. But close votes based as much on whim and group dynamics as on solid scientific information provided some tense moments for the committee and a sobering experience for observers.

In contrast to the serious discussions, the logistics of 20 scientists moving ponderously through three guideline versions printed in variorum style and governed by Robert's Rules of Order gave the meeting an undercurrent of comedy. An honest transcription of the meeting would, almost certainly, reveal the three most common phrases to have been "Where are we?" "What's the motion?" and "What are we talking about?"

At one point, the committee came close to a serious derailment. During discussions of containment categories for amphibians, insects and lower eukaryotes, a motion was made to insert a "grandfather clause" into the guidelines to allow continuation of experiments begun under the looser Asilomar levels at those same low levels now. The committee almost passed the motion until a speech by a respected colleague and observer Sydney Brenner of Cambridge saved them from a move that would surely have been regarded by many as buddyism and disregard for safety.

Brenner represents the more conservative view toward safety and containment common among European molecular biologists. At one point, he suggested that a dangerous experiment be done at NIH's high containment facility to establish a baseline for just how dangerous laboratory organisms such as *E. coli* K-12 and the ColE1 plasmid vector may be. Expectations are, at this point, that such hybrids may be far less dangerous than previously believed. "But," Brenner says, "if we do not do such a baseline experiment, we will have to take the undesirable approach of retrospective epidemiology on our laboratory workers."

The NIH committee, although finished with this draft of the guidelines, is far from relieved of regulatory duties. One committee member, dismayed by the huge task of regulating this burgeoning field, fears the committee will become, to paraphrase Edwin Chargaff, the Bishops of NIH. Subcommittees will, for example, screen all NIH grant requests and requests for changes in containment levels during ongoing experiments. Implementation of the guidelines is, in general, in a somewhat undefined state. But they will have to remain so, the committee decided, until the next meeting in March. □

Nova Cygni's curves: New twists in theory

A nova that reaches naked-eye brightness is unusual in a simple statistical sense, and Nova Cygni 1975, which exploded at the end of August (SN: 9/13/75, p. 165; 9/27/75, p. 196), was certainly that. More important, it now seems that the nova was astrophysically unusual too and may require new departures in the theory of what a nova is. This is the consensus of a variety of international studies of the nova reported at this week's meeting of the American Astronomical Society at Chicago's Adler Planetarium and in a preprint sent to SCIENCE NEWS by astronomers at the Torun Observatory in Poland.

The Polish observers, including Wilhelmina Iwanowska, A. Burnicki and A. Woszczyk, set the tone: "Nova Cygni 1975 is a very unusual nova. . . . It is really a very fast nova: The rate of decrease in its brightness in the early decline phase was about 1 magnitude per day." Other observers concur, though there is some disagreement on the total rise in magnitude. Some American speakers cite a rise of 19 magnitudes (a 40-million-times increase in brightness) or more. The Polish observers mention a pre-nova observation of the star by an unnamed Soviet astronomer that rated the object at 16th magnitude, giving thus only a 14-magnitude rise.

The nova's intrinsic (as opposed to visual) magnitude at peak was calculated at between minus 9 and minus 10. This is not an unusual peak intrinsic brightness for a nova, but, as J.S. Gallagher of the University of Minnesota puts it, Nova Cygni 1975 "was the fastest ever," both in rise and fall of brightness.

Spectra taken at various places from Torun to York, Ontario, to Minnesota show complicated changes as the nova developed that often do not conform to expectations from the usual nova theory. Gallagher, using infrared observations, finds a basic astrophysical discontinuity in the nova's development. At first it shows the characteristics of a blackbody—thermal radiation from the collection of matter that makes it up. Later, a break in the spectral appearance, especially that of the emission lines of hydrogen, lead him to suspect that the matter in the supernova or the cloud exploding off it has become ionized. The radiation is now the sort of thing that comes when two unbound charged particles approach, pass and revolve around