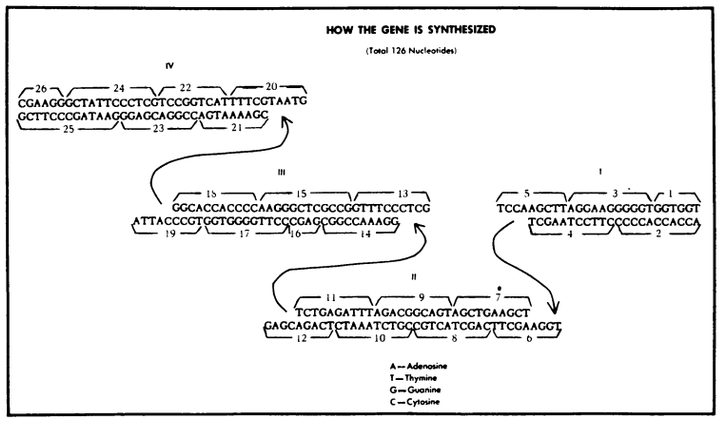
A 126-unit artificial gene

MIT scientists have synthesized the first gene with the potential to function detectably within the living cell



MIT

One of the biggest challenges in molecular biology is determining the chemistry of genes and how this chemistry orders genetic information. A major step toward this end was announced in 1970 by Nobel laureate chemist Har Gobind Khorana and his team. They were the first scientists to synthesize a copy of a real gene. The gene, found in yeast cells, orders transfer RNA to line up the amino acid alanine into protein (SN: 6/6/70, p. 547).

The artifical gene had two drawbacks though. Because the start and stop signals for the alanine tRNA gene were not known, the scientists could not attach these signals to the artificial gene. And without the start and stop signals, the artificial gene could not be made to function in a yeast cell. And even if the artificial gene could have functioned in a yeast cell, alanine tRNA activity ordered by it could not have been detected. This is because the yeast cell's natural genes activate alanine tRNA as well.

Khorana and his team have now synthesized another gene. This one is the first synthesized gene that has potential for both functioning in a living cell and for having its product detected in a living cell. The achievement was reported this week by Kanhiya Lal Agarwal, one of Khorana's colleagues at the Massachusetts Institute of Technology, at a meeting of the American Chemical Society in Chicago.


Khorana: Heads gene-synthesis team.

The gene is a copy of one present in the bacterium *E. coli* that orders tRNA to line up the amino acid tyrosine into protein. Hence the name of the artificial gene is "tyrosine transfer RNA gene." The MIT researchers have not yet completely figured out the start and stop signals on the natural gene, nor completely grafted the signals onto the artificial gene, but they have made ample progress toward this end. Once they get the start and stop signals on the artificial gene, the gene can be introduced into a bacterium via an infectious virus. Once the gene is inside a bacterium, the scientists will be able to see whether it is functional.

Khorana and his colleagues originally began synthesizing a tyrosine tRNA gene with 85 nucleotides. Nucleotides are the chemical building blocks of genes. Then Sidney Altman and John Smith of the University of Cambridge, England, found that there are 41 more nucleotides on the gene. The 126-nucleotide gene was longer than the functional tRNA gene. For some unknown reason, after the long tRNA gene is synthesized, the extra 41 nucleotides break off, creating the functional tRNA gene. So the MIT investigators set out to synthesize the longer gene.

They began building their gene by synthesizing small segments of 10 to 14 nucleotides. Each segment consisted of a complementary portion of two opposing segments of the two-stranded molecule. Thus, each segment acted as a splint to attract and hold together two opposing segments, which could then be tied together by an enzyme called DNA ligase. The scientists designed the synthesis so that these joined segments still had a left-over, single-stranded segment extending beyond the double-stranded segment. This left-over segment was used as a splint to join more segments of the gene. The scientists joined overlapping segments this way until they produced four large portions of the gene. These were joined to produce the entire gene.

As for the stop signal on the gene, Khorana and his colleagues have synthesized 24 nucleotides. They believe these nucleotides comprise a major portion of the stop signal. They are now sequencing the start signal of the gene using the natural gene as a guide.

However important the MIT team's achievements, they are but a beginning in understanding the chemistry of genes. The yeast gene that Khorana and his colleagues synthesized was 77 nucleotides long, the bacterium gene 126 nucleotides. Human genes, in awesome contrast, contain millions of nucleotides each. □

Controlling epilepsy by biofeedback

One of the most popular gadgets to come out of psychological technology is the alpha machine. Basically, it is an electroencephalograph recorder with sound or light that signals or feeds back to the user whenever a specific brainwave pattern is being produced. Using this biofeedback technique, people are attempting to learn to emit more than the normal amount of alpha waves. These waves are associated with relaxation, but not all psychologists agree there is any benefit to be derived from producing extra amounts of them (SN: 11/6/71, p. 314). What psychologists do agree on is the value and eventual importance of the biofeedback technique for brainwave control. And some researchers are now using it in

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an attempt to teach epileptic patients to produce fewer abnormal brainwave patterns.

This week at the annual meeting of the American Psychological Association in Montreal, M. B. Stearman of the Veterans Administration Hospital in Sepulveda, Calif., reported that he has used the technique successfully with four severely afflicted epileptics.

Stearman began working with cats and found that they produced a specific EEG rhythm when they are relaxed and not moving. The rhythm (12 to 16 cycles per second) comes from the sensorimotor cortex and is called the SMR. Cats conditioned to produce abnormally high amounts of SMR were found to be resistant to drug-induced seizures so Stearman attempted to find a similar SMR in humans. He is now teaching epileptic patients to change their abnormal brainwave activity and reduce their seizures by producing more of the SMR.

When patients use the biofeedback system, it rings bells, lights lights, or advances a slide on a projector every time they produce the SMR. The patients Stearman is working with did not respond to anti-convulsant drugs but after one month of training (three hours a week) they were able to produce the appropriate sensorimotor rhythms at will. After three months of training, all showed significant improvement in EEG patterns and reduction in number and intensity of epileptic seizures. One woman went from 21 seizures a year to only 7 after an 18-month training period. Another patient, a 6-year-old boy who was severely brain damaged and suffered from hyperkinesis, was considered uneducable. During 12 months of training he showed significant improvement in learning ability and a reduction in hyperactivity. He had only two seizures during the training period, but when he went off the training (but stayed on medication) his seizures became so bad that he had to be hospitalized. He improved again when biofeedback training resumed.

Stearman does not say he is curing epilepsy, but he does believe he is teaching patients to control it. He suggests that learning to produce the sensorimotor rhythms may lead to a reorganization of the diseased area of the brain. Bonnie Kaplan at the VA Hospital in Bedford, Mass., has attempted to reproduce Stearman's findings. Using similiar techniques, she has found no change in patients' EEG that could be considered the result of biofeedback training, but, she says, two or three patients did have fewer seizures. She and Stearman intend to continue the research to find exactly what in the biofeedback training controls epilepsy. □

Polywater evaporates: An insight on science

Scientists, like judges, tend to go by the principle of *stare decisis*; they rely on past results. The manner of thinking that the scientific method makes habitual tends to favor results that have stood the test of time and repeated experiment. In spite of (one might perhaps even say because of) its much touted openmindedness, scientific method contributes to a scientific orthodoxy, a body of well-accepted results that it becomes dangerous to question.

When that orthodoxy is challenged, the response of the scientific community is interesting to watch. We speak here not of such an obvious case as spirit rapping, but of a new and anomalous form of water.

Anomalous water, sometimes later called polywater, first appeared in the Soviet Union. It came to the world's attention in 1967 in a publication by B. V. Derjaguin, N. V. Churaev and N. N. Fedjakin of the Institute of Physical Chemistry of the U.S.S.R. Academy of Sciences. They had found, they said, a strange form of water that condensed in tiny capillary tubes. This anomalous water was as viscous as heavy oil and had other odd properties: for instance, it could be cooled to sub-zero temperatures without freezing into crystalline ice.

Water is a very well investigated substance, and it seemed incredible that such a weird form of it had escaped notice for so long. Yet there it was, reported by reputable chemists. It was a topic that could prove very dangerous for scientific reputations, yet it could not be ignored.

From the outset there were two main opinions. One side held that anomalous water was ordinary water in which dissolved impurities were responsible for the odd properties. The other held that anomalous water was some important new kind of structure involving water molecules.

But scientists are not supposed to proceed from dogmatic assumptions. Experiment was necessary, and in several countries it began. Experiment proved difficult. The stuff could only be made in minute quantities, which made analysis difficult. Samples could not be exchanged from laboratory to laboratory. This led to an unusual kind of scientific argument: One could detract from an opponent's results by suggesting that whatever he had experimented with was not really anomalous water.

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Derjaguin: Polywater is dirty water.

During 1968 and 1969 much experimenting was done, but hardly any results were published. Whether this was because experiments took time to analyze or because chemists hesitated to lay their reputations on the line is hard to say, but it seemed for a while almost as if the topic had gone underground.

In the middle of 1969 came a publication based on infrared spectroscopy of anomalous water that argued that the substance was a polymer built of water molecules. This was perhaps the high point of the new-structure argument. It was followed by other suggestions in the same vein but different in detail plus theoretical speculations about how such a polymer might be built up and the possibility of similar structures in other materials.

But the opponents were still at work, and they were using sophisticated methods to analyze anomalous water. They found many impurities, and at a symposium at Lehigh University in Bethlehem, Pa., in June 1970, they said so. The audience was left feeling that very clean experiments would be necessary to uphold the new-structure side.

In the end such experiments did not come forth. The history of the subject is downhill from mid-1970 on. Now, finally, anomalous water has been buried by its discoverers. Derjaguin and Churaev report in the Aug. 17 NATURE that they now believe that the strange properties of anomalous water were due to impurities. This reverses their original opinion that challenged the scientific orthodoxy. The important thing is not so much the vindication of the orthodoxy as that the scientific method led them to change their minds.