Functioning Artificial Gene Synthesized

For nine years now, Har Gobind Khorana and his team at the Massachusetts Institute of Technology have plodded, persevered and performed numerous biochemical gymnastics to achieve, finally, one of molecular biology's greatest feats. They have synthesized a gene that is able to function within a living cell.

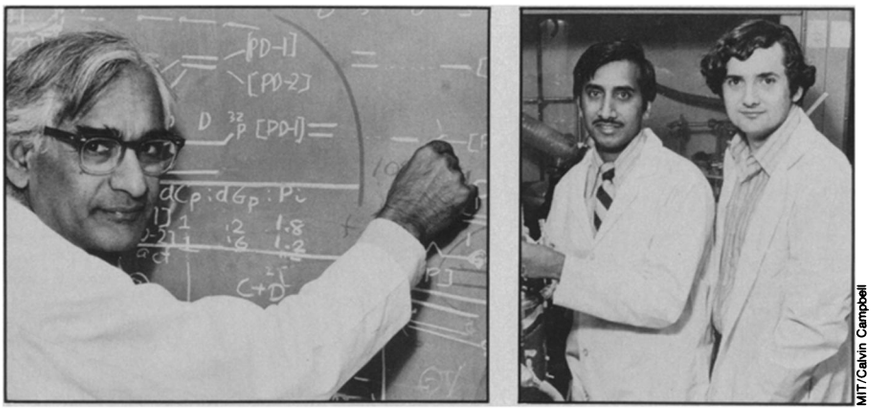
The achievement was reported this week at the meeting of the American Chemical Society in San Francisco by two of the MIT team, Ramamoorthy Balagaje and Hans-Joachim Fritz. Other team members include Eugene L. Brown, Robert G. Lees, Takao Sekiya, Tatsuo Takeya, Michael J. Ryan and Hans Küpper.

Probably the most significant aspect of the achievement is that it vindicates the entire edifice of modern genetics. It proves that molecules of DNA are indeed the genetic basis of life, not solely a theoretical construct. The achievement also sets the stage for better understanding gene action and eventually synthesizing human genes to correct genetic diseases.

The gene the MIT team synthesized is a copy of one naturally found in the bacterium *E. coli*. Called the tyrosine transfer RNA gene, it makes a molecule known as tyrosine transfer RNA. Tyrosine tRNA then finds a free-floating amino acid, tyrosine, in the bacterium and transfers it to a ribosome where it will combine with other amino acids into a protein. Nine years ago the nucleotide sequence of this gene had been unraveled. That set the stage for the MIT team's attempt to synthesize it.

The scientists used purified, commercially available nucleotides to assemble the gene nucleotide by nucleotide in the same order as those in the natural tyrosine tRNA gene. They chemically activated sugars in the nucleotides so that the nucleotides would join together properly. They temporarily blocked certain chemical constituents on the bases of the nucleotides so that they would not hook up erroneously to nearby sugars. Two nucleotides were joined with two nucleotides, four nucleotides with four, and so on until some 10 or 15 were strung together. After each nucleotide segment was made, the scientists had to purify it using a rapid, high-pressure liquid chromatography method developed by Fritz. Finally, when they had obtained some 40 purified nucleotide segments, they used enzymes to link the segments into a double-stranded DNA molecule corresponding precisely to the natural gene (SN: 9/1/73, p. 132).

Then came another, equally formidable challenge: Not only stringing together nucleotides to make the gene's start-and-stop signals, but determining which nucleotides the signals consisted of in the first place. Ultimately, the team announced that they had sequenced the start-and-stop signals, known as the promoter and terminator (SN: 9/21/74, p. 180). This announcement proved to be somewhat premature because the promoter turned out to be longer than initially estimated, 52 rather than 29 nucleotides.



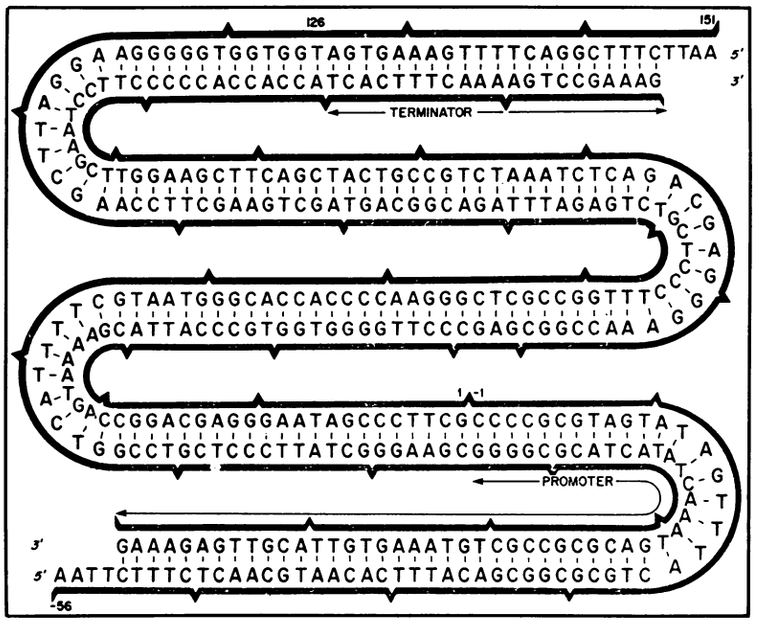
Khorana, Balagaje and Fritz: Complete nine-year effort to make a fully functional gene.

Now, finally, the team has completed the total synthesis of both the promoter and terminator, linked them to the gene, introduced the total package into an *E. coli* cell and shown that the gene functions. The evidence that the gene works is somewhat indirect. They introduced the gene package into the DNA of a virus that lives in *E. coli*. Before the gene was introduced, the virus was unable to grow; after the gene was introduced, the virus was able to grow. But as Fritz points out, the gene was "expressed there as if it were part of the genetic information in the bacterium itself."

The accomplishments were hailed this week by many leading molecular biologists. Joshua Lederberg, Nobel laureate geneticist at Stanford University, said the fact that the gene works in a living cell is intellectually gratifying because it supplies the ultimate proof that "the whole theoretical edifice of DNA genetics" is correct. The synthesis, he says, is also "an extremely powerful tool to give us information at the actual chemical level about how genes function."

James D. Watson, who won a Nobel Prize in 1958 for helping determine the double helical structure of the DNA molecule, called the work "a pretty piece of biochemistry."

Last year, Herbert Boyer of the University of California Medical School in San Francisco synthesized a strip of DNA, not an entire gene, that worked in a cell. He predicts that "the whole technology of synthesizing genes is going to blossom



Structure of the synthetic E. Coli tyrosine transfer RNA gene synthesized by the Khorana group. Segments between points were linked chemically, then joined enzymatically to form the entire DNA double helix.

quite rapidly now."

However, this may not necessarily be the case, according to Ryan at MIT. His group is the only one in the world now working on synthesizing functioning genes, although there are some others who are synthesizing nonfunctioning genes and strips of DNA that can function (SN: 12/13/75, p. 372). Ryan says that rapid progress in the synthesis of functioning genes is hindered by the the fact that "it is a tremendous amount of work." If it took the MIT team almost a decade to synthesize a gene only 207 base pairs long, how long would it take them to synthesize a gene 1,000 to 3,000 base pairs long, the size of a human gene, not to mention to unravel the nucleotide sequences of start-and-stop signals and to synthesize them as well? Unless there are some unexpectedly dramatic advances in biochemical methodology, he says, the synthesis of human genes won't take place for many more years. "You are talking about something my grandson might be doing," he says.

Fritz agrees: "Although it is possible that manmade genes may be used to correct genetic defects, that could be very far in the distant future." Still, he and other molecular biologists are confident that the synthesis of human genes will eventually be accomplished, and when that day finally comes, the technique will be used to help people with genetic diseases. The gene that makes human hemoglobin, for instance, might be synthesized, then injected into sickle-cell anemia patients, thereby replacing their defective hemoglobin and curing them of the disease. Or the gene that makes human insulin might be synthesized and, with the help of recombinant DNA techniques, inserted into the DNA of bacteria. The gene would then make insulin which could be harvested and used to treat diabetics.

Meanwhile, the MIT team will try to learn more about gene expression and how alterations in that expression might give rise to genetic diseases. As Fritz envisions, "We can now integrate specific changes in the structure of the gene and look at potential changes in its function. This has enormous implications for genetic defects in hereditary diseases where something in the gene control goes wild, and we don't know what parts of the gene are wrong." □

Acetylene in interstellar space

So many chemical compounds have been found in the gas and dust clouds of interstellar space that astronomers are beginning to feel that any compound they would like to think of could be found there if the means of finding it were available. Up to now, astronomers have mostly used the microwave range of the radio spectrum in their searches and so have been limited to compounds that have prominent resonance lines in that range. Now the discovery of acetylene (C_2H_2) by workers at the Kitt Peak National Observatory adds the possibility of systematically exploiting the infrared range of the spectrum.

The work was done by Don Hall and Stephen Ridgway of Kitt Peak with the assistance of Robert Wojslaw of Kitt Peak and Susan Kleinmann and Doreen Weinberger of the Massachusetts Institute of Technology. The acetylene was found in an area of the constellation Leo known as IR + 10° 216, a region of shells and an extended cloud of matter surrounding a very old carbon-rich star. Presumably the shells and cloud are matter thrown off by the star in its death throes.

The observation was made with Kitt Peak's 4-meter telescope, an instrument for visible-light observation, but also capable of infrared studies, and a Fourier transform spectrometer, which separates the wavelengths of the infrared and records the spectral characteristics of the source. This is a very new instrument.

Another important point is that the acetylene discovery was made in the middle of the afternoon. The stars cannot be seen clearly in the daytime because interference caused by sunlight scattering in the atmosphere blocks out their images. Infrared is much less subject to this scattering. So infrared observations can be done in the daytime, and the 4-meter Mayall telescope, one of the world's largest, now equipped with its Fourier transform spectrometer for infrared, can be kept working 24 hours a day. □

Occam's Razor: Viking's thin edge

William of Occam may turn out to be the unofficial patron saint of the Viking mission to Mars. *"Pluralitas,"* wrote the 14th-century Franciscan ranked by some as the last major medieval philosopher, *"non est ponenda sine necessitate."* Do not invent more hypotheses than are necessary; or, as it has come to be applied in more recent times, the simplest explanation that will fit the facts is probably the best bet. The idea has come to be known as Occam's Razor, and the man himself used it largely to shave off as unnecessary many of the split-hair, interfactional theories that continually rattled metaphysical thought and church-state relations throughout his life and times.

In science, perhaps even more than in theology, the Razor's role is often that of caveat, rather than of more general *modus operandi*. If you're having to stretch your present line of reasoning too far to explain what you're seeing, even if you have not yet been proved wrong, perhaps it's a sign that an alternative approach is closer to the truth. In Project Viking, with Mars providing new examples of the unexpected at every turn, old William's spirit is just starting to work out with the strop.

The most obvious example, though it's true in other fields as well, is the search for life. Two of the three experiments in the elaborate biology instrument are showing almost exactly what they ought to be showing if there are little Martians in there doing their metabolic thing. One problem is that the results suggest similarly energetic responses to two diametrically opposed chemical environments. A more fundamental issue, however, is that Viking's "biology" experiments are *not* designed to detect "life," whatever that is. They watch only for evidence of certain, extremely specific reactions that terrestrial experience says are usually among the list of life *processes*. It is the scientists' burden to push as close as possible to the knowledge of whether these reactions also represent life on Mars.

The first step—in fact it's virtually the only step—is to rule out all the possible nonbiologic ways of producing the same reactions. But for a virtually unknown planet, before they can be ruled out, they must first be conceived. And some of the proposals have been pretty exotic.

If Mars were more familiar—like earth, for example—Occam's Razor would already have been slashing away with abandon, inspired by a fresh crop of theories ranging from the plausible to the house-of-cards. But it takes time to draw enough useful guidelines to do a little cutting. A long, exponential growth curve in the labeled-release experiment, for example, would rule out a lot of present chemical possibilities on the kinetics of the release rate alone. (The LR instrument began looking at its latest sample on Aug. 28, and will continue to do so until solar conjunction shuts off communications with the lander early in November.) Occam's Razor opts for the simplest explanation that fits all the facts, and so far, the facts are simply too few.

Nonetheless, it's a useful tool. Viking's biology package may provide extremely strong evidence of life, perhaps even leading to a consensus, but it will never be able to provide absolute, 100-percent proof. Nor will a biologically oriented "wet chemistry" instrument (which would seek optically active amino acids), nor a multiple-reagent "integrated" biology instrument being considered for possible post-Viking missions. Even the various proposed automated missions to return a Martian soil sample to earth ("Andromeda Strain" considerations are a separate problem) could be less than conclusive, since the sample would be vibrated, exposed to alien materials, cut off for months from its native sunlight and deprived of its usual atmospheric interactions. The razor may still be needed to separate the reasonable from the merely