

On the brink of a functioning artificial gene

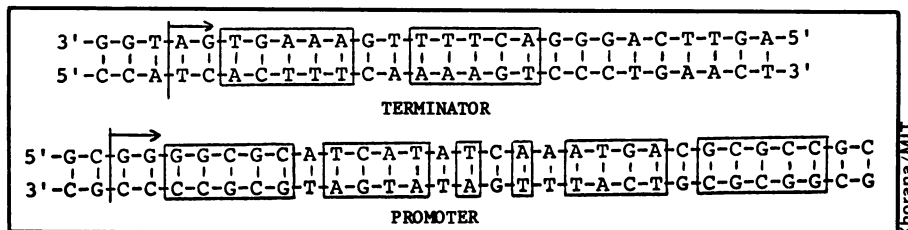
A year ago Nobel laureate molecular biologist Har Gobind Khorana announced the synthesis of a 126-unit artificial gene with the potential of directing the production of tyrosine transfer RNA within a bacterial cell (SN: 9/1/73, p. 132). But there were two elements missing from the gene—the “on” switch (promoter) and the “off” switch (terminator).

Last week at the meeting of the American Chemical Society in Atlantic City, Khorana and colleague Rama-Moorthy Belagaje, both from the Massachusetts Institute of Technology, announced completion of the synthesis of both the promoter and terminator regions. They now are linking them chemically to the gene and soon may create the first functioning man-made gene.

By following the sequence of base pairs on the region of bacterial DNA that directs the synthesis of tyrosine transfer RNA production, they determined the order of 29 nucleotides (the chemical building blocks of genes) in the promoter region and 23 in the terminator region. When the artificial gene is inserted into the existing genetic code of a bacterial cell, the end product should be the loading of tyrosine onto growing protein chains.

The team has developed a chemical method for linking nucleotides in sequence instead of using polymerizing enzymes. Enzymes will form sequences based only upon existing DNA codes, Belagaje says, but entirely new code sequences can be formed chemically. “With chemical joining techniques, we can study gene function by designed chemical change. After we get an artificial gene functioning, we can switch the sequence in the control region and see what results,” Belagaje says.

Researchers in several other U.S. laboratories are also attempting to answer what Khorana calls the “central question in modern biology”—how does a cell control the transcription of its DNA into the right proteins at the right time and place? Biochemist James E. Dahlberg from the University of Wisconsin at Madison, reported on a new kind of molecule that is required for copying genetic information in tumor viruses. He found that tryptophanyl transfer RNA, a normal cell component, acts as a special “primer” in the reverse transcription from a tumor virus RNA to bacterial DNA. The molecule functions in protein synthesis in the normal cell,



Order of the building blocks in the “on” and “off” switches of a man-made gene.

but is “pirated” by the virus and packaged into virus particles for reinfection of other cells, Dahlberg says.

Another important piece in the expanding picture of gene control was presented by John Abelson of the University of California at San Diego. Working with the human intestinal bacterium *Escherichia coli*, he has determined the nucleotide sequence of the control region of the lactose operon system. This system was first characterized by Jacques Monod at the Institute Pasteur in Paris and has become the classic model of the influence of nutritional environments on gene expression. Abelson compared his nucleotide se-

quence with Khorana’s, but the sequences were different, dampening the hopes for finding a universal on-off sequence.

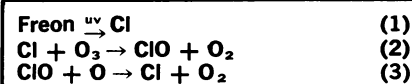
About gene research in general, Khorana says, “Now that we have the methods worked out” for manipulating nucleotides, building and controlling artificial genes “is no longer regarded as so formidable.” But it must be remembered, he says, that researchers are still working with simple bacterial gene systems. Before genetic engineering can be used to correct inborn human diseases, human genes must be studied. And they contain millions, not hundreds, of nucleotides. □

Freon: Destroying the ozone layer?

A fascinating paradox has surfaced regarding man, ozone and the atmosphere that adds an ironic twist to the story of technological advancement. Researchers have noted during the past two decades that nitrous oxides and hydrocarbon pollutants building up in the lower atmosphere are acted upon by sunlight to produce ozone (O₃). High levels of ozone, in turn, cause respiratory problems and kill plants.

Now, it seems, Freon and other fluorocarbon pollutants in the upper atmosphere may be removing ozone, which acts as a protective layer against harmful ultraviolet light. (Freon is a DuPont tradename but is used as a generic term by many scientists.) By polluting his own air, man may be creating too much ozone below, too little above and possibly deleterious effects from both conditions.

Freon 11 and 12 (CFCl₃ and CF₂Cl₂) are inert compounds used widely as aerosol propellants and refrigerants. The United States will produce and use about 800 million pounds in 1974. Because they are chemically inert, they float up through the lower atmosphere unchanged and are suspended about 20 miles above the earth.



Model for destruction of ozone.

John W. Swinnerton, a chemical oceanographer at the Naval Research Laboratory in Washington, reported on the problem at the annual meeting of the American Chemical Society in Atlantic City last week.

While monitoring levels of other atmospheric pollutants over remote ocean sites during the early 1970’s, researchers noticed persistent indications of freons in the upper troposphere (about seven miles above the earth’s surface). Monitoring specifically for fluorocarbons, Swinnerton and others found that “even in remote areas of the Pacific and Atlantic, the freon concentrations are climbing rapidly.” In 1972, on a cruise from Los Angeles to the Antarctic, he found an average of 61 parts of freon per trillion parts of air. In 1973, 85 parts per trillion were detected over the Atlantic, and in January of this year, Swinnerton found about 120 parts per trillion in the Arctic air near Spitsbergen. Freons definitely are