On the brink of a functioning artificial gene

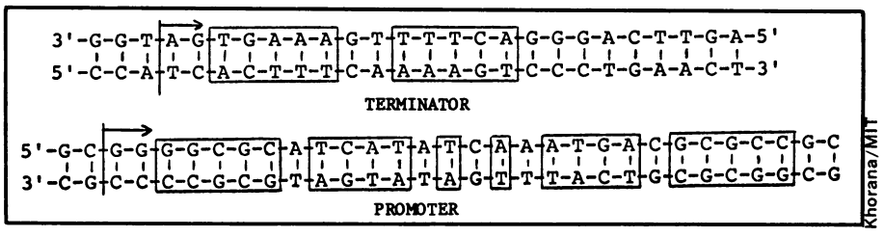
A year ago Nobel laureate molecular biologist Har Gobind Khorana announced the synthesis of a 126-unit artificial gene with the potential of directing the production of tyrosine transfer RNA within a bacterial cell (SN: 9/1/73, p. 132). But there were two elements missing from the gene—the "on" switch (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 sequence 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 (O_3). 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 ($CFCl_3$ and CF_2Cl_2) 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.

$$\text{Freon} \xrightarrow{uv} Cl \quad (1)$$
$$Cl + O_3 \rightarrow ClO + O_2 \quad (2)$$
$$ClO + O \rightarrow Cl + O_2 \quad (3)$$

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

collecting in the upper troposphere, he says, at a rate approaching the nine percent worldwide increase in production per year. What is not yet known is whether, and to what extent freons are collecting in the stratosphere (the upper atmosphere, above seven miles from the earth's surface), the proposed breakdown site.

Frank S. Rowland and Mario J. Molina, physical chemists at the University of California at Irvine, have proposed a model for freon breakdown and ozone destruction. It is based on a similar reaction between nitrous oxide and ozone in the lower atmosphere. First, they propose, freons in the stratosphere absorb ultraviolet light in the 1,750 to 2,200 angstrom range, and chlorine is liberated. The liberated chlorine atom in turn attacks ozone, breaking it into oxygen. Each chlorine atom can remove thousands of ozone molecules from the stratosphere in this way, Rowland predicts.

Concentrations of fluorocarbons can be expected to reach 10 to 30 times their present levels if production continues to increase at the current nine percent per year. The result would be the destruction of 10 percent of the stratospheric ozone layer within 50 years, Rowland says. He has already calculated a one percent reduction in stratospheric ozone—a reduction that could result in about 8,000 additional cases of skin cancer this year, according to National Academy of Sciences statistics on skin cancer. After 50 years (and 10 percent ozone destruction) that number could be 80,000 per year.

Two physical chemists from the University of Michigan at Ann Arbor will report computer calculations on ozone destruction in the Sept. 27 SCIENCE. Ralph J. Cicerone and Richard S. Stolarski predict that freons will cause a "marked reduction" in stratospheric ozone "exceeding that predicted for a 500-plane SST fleet" by 1985 or 1990. Their model is more pessimistic than Rowland's, predicting the same 10 percent reduction in stratospheric ozone but after much less time and with lower stratospheric fluorocarbon levels.

Potentially more dangerous than the threat of increased skin cancer, Rowland says, is the threat of climatic changes after ozone destruction. "Ozone is the heat source for the stratosphere, and if it is thinned, it allows for more infrared rays (heat) to pass through to the troposphere and thus to shift atmospheric temperatures." Not enough is known about the structure of the stratosphere and its climatic interactions, but heat shifts could cause wind shifts and changing global climatic patterns, he says.

Current methods are inadequate for measuring fluorocarbon concentrations and ozone destruction, Rowland said when challenged for experimental evidence by DuPont chemical company spokesmen during a press conference. (DuPont is the largest U.S. producer of fluorocarbons.) The chemical breakdown sequence is only a model at this time, and Rowland's one percent decrease in stratospheric ozone was calculated by chemical rates and not by observation or direct testing. It is not known, for example, whether fluorine atoms also are photodissociated from freons and destroy ozone. Other ozone-destroying chlorine compounds might also exist in the stratosphere that are not detected with current analytical methods.

But work should not be suspended until new methods are developed, he says. "There is enough information available to persuade us that the eventual risk is large and that all aspects of the problem should be examined on a broad scale." Society must begin to assess the benefits of fluorocarbon aerosols and refrigerants compared with their potential risks to human health and world climates, Rowland says. "My own opinion is that the advantages of the former are not worth the risks of the latter." □

Ford signs solar heating bill

President Gerald R. Ford has signed into law the Solar Heating and Cooling Demonstration Act of 1974, designed to introduce solar energy into American homes and businesses over the next five years. The bill represents a compromise among several different House and Senate versions, arrived at following a year of intense argument over what agency should handle the project and prolonged handwringing over previous failures to exploit so attractive an energy source (SN: 4/13/74, p. 242).

In the final version, the National Aeronautics and Space Administration (NASA) is given responsibility for procuring and developing the necessary technology to heat and cool homes and office buildings at a reasonable price, and the Department of Housing and Urban Development (HUD) is charged with demonstrating the effectiveness of such systems under a variety of conditions. In practice, this means that NASA will try to cut the cost of solar collectors from their present $5.50 a square foot to around $2 a square foot—the cost level considered necessary for solar energy to compete nationally with alternative sources. Developers will also have to concentrate on increasing reliability so that when the units are eventually marketed, companies can guarantee their performance for 10 to 15 years. Meanwhile, HUD will subsidize the installation and testing of trial units on private homes and other buildings throughout the country. The National Bureau of Standards will work with HUD to develop performance standards and testing procedures.

Congress has authorized $60 million for the five-year project, which does not affect ongoing basic research on solar energy being conducted by the National Science Foundation. A separate bill, to give as much as a billion dollars for research and development of solar energy over the next five years, passed the Senate this week. A recent study sponsored by NSF indicated that if the federal Government would undertake an initial incentive program, private industry would rapidly show increased interest. With such participation, the study concluded, by the year 2000, some 4 million buildings may be solar equipped (SN: 6/29/74, p. 412). Until now, the solar industry has been dominated by small entrepreneurs using highly experimental designs, while major companies have taken a "wait and see" attitude (SN: 2/2/74, p. 69).

The two lead agencies are now expected to draw up a tentative working plan, which they will submit to the Congress within four months, together with requests for specific appropriations. Since simple heating systems have progressed further toward marketability than the more involved heating-cooling systems, the time-scales proposed for the two projects are expected to be substantially different. Other agencies are also expected to become involved in the effort. The Department of Defense, for example, is expected to work with HUD on installing solar units on military bases.

Besides working out the technical bugs in present solar systems, the five-year demonstration project is designed to boost public acceptance of solar energy. In part this will be accomplished simply by distributing working models around the country, so that soon the average American will personally have seen solar energy in action (supposedly, people are most conservative when it comes to buying homes, and the home building industry fears the acceptability of "unsightly" solar collectors). A more subtle problem will be changing the current financing arrangements that put solar systems at a disadvantage because of their large initial cost. A major educational program will aim at introducing the concept of "life-cycle" costs, which will show homeowners that solar systems theoretically increase the resale value of their houses. □