Gene parts sandwich surprise segments

Imagine finding several lines of a sonnet or a page of a thriller in the middle of a recipe for spaghetti. Researchers were just as surprised to find in several types of cells stretches of DNA that do not contribute to the structure of the gene's product. On the basis of earlier experiments with bacteria and their viruses, the sequence of nucleotides in a gene was thought to be a linear, uninterrupted representation of the gene product. One could look at the order of the nucleotides and predict the amino acids in a resultant protein or the sequence of nucleotides in resultant RNA molecules. But, as researchers discovered in studying the attachment of "leader" sequences to genes coded some distance away on the chromosome (SN: 7/30/77, p. 71), organisms higher than bacteria and blue-green algae seem to do things quite differently.

"It was horrifying to find this type of insertion in a producing cell," says Walter Gilbert of Harvard University. Other molecular biologists are calling this discovery the most shocking result to come out of the new methods that allow for analysis of nucleotide sequences of genes.

Since the first reports of "intervening" sequences about six months ago, an important question has been whether the genes in which these sequences appear are actually working. At any time many genes in a cell may be turned off, both because there are numerous copies of some genes in a single cell and because a cell does not continuously make all the many products coded by its DNA. Some researchers have even proposed that the intervening DNA is the damper that prevents a gene from operating.

Now experiments by Howard Goodman, Maynard Olson and Benjamin Hall clearly demonstrate an intervening sequence in an almost certainly functional yeast gene. Called a suppressor, the gene contains a mutation that compensates for a fatal mutation previously introduced into the yeast. The suppressor gene must operate for the cell to survive. Without the suppressor, the yeast would recognize a stop signal in the midst of an essential protein and produce a nonfunctional product. With the suppressor, one type of transfer RNA mistakes the stop signal for the tyrosine amino acid code and thus produces a functional protein, similar enough to the original gene product to save the yeast. "You know the gene has to be active, because it is the only one with the ability to suppress," Hall says.

The researchers, working at the University of Washington in Seattle, determined the nucleotide sequence of a yeast suppressor gene, one of eight yeast genes for the transfer RNA molecules that insert tyrosine into proteins. They found a section of 14 nucleotides in the middle of that gene that were not represented in the product transfer RNA. Olson reported the finding at a recent meeting in Cold Spring Harbor, New York. The investigators also determined the nucleotide sequence of the gene in the normal, non-suppressor yeast and that of two other tyrosine transfer RNA genes. "Each has a similar piece of DNA in the middle," Hall says.

Mouse embryonic genes provide another recent example of an intervening sequence. Susumu Tonegawa of the Basel Institute for Immunology in Switzerland has been examining the gene for the immune system protein immunoglobulin. His earlier evidence indicated that two separate genes code for continuous regions of that protein, and that the genes rearrange during development (SN: 12/11/76, p. 372), Tonegawa, working with Gilbert, Ora Bernard, Nobumichi Hozumi, Allan Maxam and Richard Tizard, determined the sequence of the gene for the "variable" region of the immunoglobulin molecule. He found an intervening stretch of 93 nucleotides within the gene. In this case the intervening sequence is in the DNA coding for a precursor protein portion that is cut off to form the final product. Intervening sequences were first reported in genes of fruit flies by David Hogness and Raymond White at Stanford University and are expected to be reported soon in mouse hemoglobin genes. However in none of these cases can the scientists be certain the genes analyzed are functional.

Most of the researchers hesitate to speculate on the role of the intervening sequences. Gilbert suggests that the existence of these areas speeds up evolution. If the sequences are copied into messenger RNA, where they would loop out and be trimmed off before the RNA is translated into protein, a single change in a nucleotide could alter what loops form. Such a change could take out or insert many amino acids, rather than just replace one amino acid, Gilbert says. "If a single mutation can produce grosser changes in protein, evolution would have a faster way of searching over new structures," he explains.

If this phenomenon of intervening sequences is so common among higher organisms, why was it never seen in bacteria? The answer might just be chance; even in bacteria only a few genes have been sequenced completely. Gilbert offers another explanation. In bacteria the chromosomes, rather than being confined to nuclei, are in contact with the cells' protein-making components. Messenger RNA is immediately used as a template for protein synthesis, even as it is being formed. Therefore, there would be no way to ensure that the proposed loops of messenger RNA would be clipped out before protein production. In higher organisms such modifications could occur while the messenger RNA is isolated in the nuclei. □

Tobacco protein may lead to heart disease

Health officials have known for years that smoking cigarettes plays a major role in progressive heart disease. Comparisons of case histories have shown that heavy smokers run a much higher risk of sustaining heart attacks than nonsmokers. More recently, autopsies have revealed that persons with long smoking histories tend to have more severe arteriosclerosis, a hardening and thickening of the arteries, than the general population. But these strong statistical implications only raise more questions: How do molecules found in smoke act to alter tissues? What is the physicochemical mechanism that induces pathogenic changes?

Now, two Cornell University researchers may have isolated the first substantial clue. They report in the August issue of THE JOURNAL OF EXPERIMENTAL MEDICINE that they have identified a small protein, rutin, that triggers the body's blood-clotting system. In so doing, rutin may be the catalyst in a chain of metabolic events that leads to the scarring and occlusion of arteries, both of which represent prime characteristics of heart disease.

According to Carl G. Becker and Theodore Dubin, experimental pathologists at Cornell University Medical Center, rutin is found in both tobacco leaves and cigarette smoke. The researchers say their tests show that rutin activates a blood component called Factor XII, which in turn initiates a series of enzyme reactions that cause blood platelets to coalesce, or clot.

Exactly how blood clotting may affect artery walls and heart muscle is not well understood, but Becker suggests one possible mechanism. Clotting may lead to a build-up of clotted blood that adheres to artery and heart walls. Becker told SCIENCE NEWS that blood platelets may form a matrix for connective tissue cells to grow on. This process, called "organization," may serve to partially block vascular passageways—raising blood pressure and increasing the work load on the heart.

Another possibility, says Becker, is that "activation of the blood-clotting system may trigger Brady-kinin, [a generic term for] a number of polypeptides which are inflammation mediators." Becker says Brady-kinin are known to alter membrane permeability, cause physical pain and attract macrophage activity, thereby creating a pathological condition that could alter artery efficiency.

Becker and Dubin came upon the discovery of rutin while testing the long-standing theory that chemicals in the tobacco leaf might cause chronic allergic reactions that then lead to heart and lung diseases. The researchers isolated a com-