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-

plex sugar-protein, or glycoprotein, that caused allergic reactions in 12 of 31 volunteers who were injected with it.

Closer chemical analysis revealed that the rutin protein was attached to the glycoprotein found in the leaf, and was also present in cigarette smoke. Rutin can be found in several vegetables, including eggplant, green peppers, potatoes and tomatoes. Investigators doubt that its existence in these foods is dangerous, however; more likely, the protein gains entry into the blood stream by means of the oxygen-transfer system in the lungs rather than through intestinal absorption.

Becker and Dubin say their results indicate that rutin could damage the heart and blood vessels by initiating blood clotting. In steady doses, such as those that occur with habitual smoking, the foreign protein could also aggravate continuous antigen-antibody formations in the lungs. The end result would be clotting, lesions, fibrous growths and perhaps mutant cells. □

Genetic markers may point to cause of RA

Certain "genetic marker molecules" occur much more often in rheumatoid arthritis (RA) sufferers than in nonsufferers, a University of Texas researcher reports. This finding gives new life to the recently discredited theory that the more than three million Americans afflicted with RA may have inherited at least a tendency to develop the disease.

There has always been an undercurrent of suspicion that RA represents a mistake of the genetically-determined immune response. Certain persons, the theory goes, inherit a predisposition to miscue their own defense mechanisms. The body mistakenly attacks itself, with its joints becoming sore, stiff and inflamed.

This "self-sensitization" concept was reinforced in 1974, when University of Washington researchers described an "immunoglobulin G factor"—found in 70 percent of all RA victims—as a complex of molecules that have forgotten their roles (SN: 4/6/74, p. 181). Each molecule, the report said, acts dually as antigen and antibody; each has a perverse affinity for the other, instead of for foreign chemical antigens. It was, as one observer described it, an incestuous relationship.

That inbreeding metaphor was not lost on epidemiologists, who reasoned that if RA were a genetically-determined immune response, then isolated populations might show the disease flourishing throughout specific family trees. A highly publicized study of the Pima and Blackfoot Indian tribes, however, flatly refuted the theory; the incidence of RA did not differ significantly from one family to another. Experimentally, the gene theory took a back seat to approaches that stress environmental factors.

Now, the report from the University of Texas Southwestern Medical School in Dallas seems to bring the thinking on RA back full circle. Peter Stastny, associate professor of internal medicine, reports he has found molecules on the surface membranes of B-lymphocytes and macrophage cells. These genetic markers, he says, occur in significantly higher proportions of RA victims than of control groups without the disease. The presence of these glycoprotein markers is determined by an area on a particular chromosome known as the "HLA-D region," which is known to have a great deal of control over the human immune response.

Fifty-eight percent of the adult RA patients Stastny tested had the HLA-controlled marker known as DW4, while only 16 percent of a control group of disease-free adults had it.

One investigator who has confirmed Stastny's findings, Hugh McDevitt of Stanford University, believes these molecular signposts may lead to diagnostic tests that can identify those people who will eventually develop the disease.

But Stastny does not believe a marker found in about one-half of all RA victims and 16 percent of those without it is specific enough to have much value. He thinks, however, that his evidence of a genetic factor will serve as a touchstone and a catalyst for new conceptual approaches to understanding the disease. □

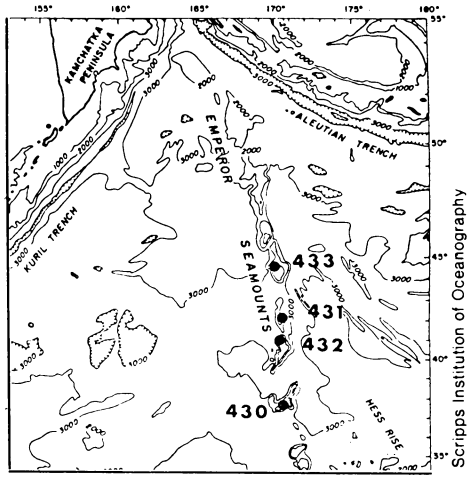
Emperor Seamounts: Hotspot candidates

In the near decade and a half since it was proposed, an idea generally known as the "hotspot theory" has achieved a highly visible position among views of how island chains such as the Hawaiian group are born. As one of the earth's crustal plates moves across a fixed source of heat beneath it, a succession of volcanoes are generated, each one sliding away with the plate to leave room for the next. The volcanic island chains, in other words, are tracings of the plate's wanderings, sketched on the crust from below.

The classic example usually cited is the Hawaiian Islands, which have yielded data in various studies in support of the hotspot theory. Not every island chain seems to fit the expected criteria, however. In 1973, for instance, the Line Islands in the Pacific were sampled by the research vessel Glomar Challenger as part of leg 33 of the Deep Sea Drilling Project (SN: 1/12/74, p. 22). Instead of revealing that the southernmost islands—nearest the supposed hotspot—were substantially younger than the northern ones, the samples showed that volcanic eruptions along the chain apparently stopped during the same period, about 80 to 85 million years ago.

Two U.S. Geological Survey scientists, Everett D. Jackson and Herbert R. Shaw, subsequently proposed an explanation nearly the opposite of the upward-heating hotspot idea. They suggested that a localized "downwelling" of heat might have been produced by friction between the moving crustal plate and the asthenosphere beneath it, leading to a melting period during which heavy materials such as iron would descend and create "gravitational anchors" to hold the viscous influx in place while the plate drifted through.

The two hypotheses need not fight to the end, however; there is room on the earth for both—and examples. The most recently completed leg of the DSDP, leg 55, has probed a string of submerged volcanic rises known as the Emperor Seamount chain, which turn out to fit the hotspot theory's conditions very



Drilling sites among Emperor Seamounts.

nicely. The Emperor Seamounts seem to be a northern continuation of the Hawaiian chain, forming a continuous (though partially submerged) mountain range some 6,500 kilometers long, formed over a period of about 70 million years.

One of the Emperor group, for example, is Suiko Seamount, from which the Glomar Challenger extracted a core sample that has preserved evidence of more than 70 successive lava flows. By analyzing the magnetic polarity of the earliest of these flows, leg 55 scientists headed by Jackson and by Itaru Koizumi of Japan's Osaka University were able to determine that the Suiko Seamount was born about 25° south of where it is today. This supports the hypothesis that the peak was carried northward by the movement of the Pacific crustal plate, moving it away from the hotspot that had spawned it.

Leg 55 also produced considerable evidence that at least some of the Emperor Seamounts, now 2 km or more beneath the waves, used to extend above the surface. There are traces of coral, for example, that have since been topped by sediment containing fossils of creatures that lived in water too deep and cold for coral to grow. □