Evolution: The Bottom Line

A new concept of plant and animal genes offers novel possibilities for exploring the mechanics of genetic variability

BY JULIE ANN MILLER

Changes, additions and deletions in the genetic material allow organisms to evolve, whether from white to black or from gills to lungs. As biologists learn more and more about the workings of the genetic code and its deciphering machinery, they are drawn to speculate about the role each operation plays in the evolutionary process. They also ponder how those genetic workings themselves evolved.

As we are not the final product of evolution, so our answers cannot be expected to be the final answers, Philip Leder of the National Institutes of Health wisely cautioned at a recent seminar. Tentative answers, however, are coming in at a rapid pace. Recent advances in analyzing genes at last allow some of the evolutionary speculation to be tested. And those same techniques lead to more speculation.

Three techniques developed in the past few years have contributed to our understanding of genetic material and its operating mechanisms. With recombinant DNA techniques, scientists are able to produce and study specific stretches of DNA. Rapid sequencing methods permit scientists to read the coding elements of a stretch of DNA, just as a cell does (SN: 4/2/77, p. 216). Finally, a "hybridization" procedure, in which a stretch of DNA lines up next to an RNA message, shows what portions of the genetic material actually direct synthesis of a protein.

Silent regions interrupt many genes

One surprising discovery of such investigations has shaken the concept of the genetic blueprint itself. All the previous studies of bacteria suggested a simple, linear scheme: A length of DNA produces a length of messenger RNA, which directs the synthesis of a protein. Scientists in several laboratories, however, now find that in many animal genes, the DNA that codes for a single protein is interspersed with lengths of apparently unrelated "silent" DNA (SN: 10/1/77, p. 214).

It appears that in the genes of higher organisms one or more segments are clipped out of the messenger RNA as it carries the genetic instructions from the cell nucleus to the protein-making machinery in the cytoplasm. Specific enzymes in the cell recognize a signal coded into the messenger molecule. The signal says: "cut here" or "reattach here."

"When I came to California in September 1976, I had no idea that a typical gene might be split into several pieces and I doubt if anyone else had," Francis Crick recounts in the April 20 SCIENCE. "Even though the experimental evidence is still very patchy, it is now universally accepted that a gene in a higher organism, coding for a protein, may have other base sequences interspersed within it."

The discovery of these intervening sequences immediately answered two questions that had worried biologists. They had wondered about the significance of long molecules of messenger RNA that appear in a cell's nucleus. Now it is clear that those molecules are the RNA as it is made from its DNA template, before the silent regions are cut out of it.

Another puzzle had been the unexpectedly large amount of DNA found in cells of plants and animals. The amount is far greater than that required to code for all the genes' products. Now biologists believe that the excess DNA is the intervening sequences. Walter Gilbert of Harvard University estimates that there is five to ten times more silent DNA than coding segments.

This new twist in our understanding of the contents of the double helix raises at least two evolutionary questions. When and how did the interspersed segments arise? And do they themselves play a role in the continual changes and rearrangements of genes during the development of all life? According to one view, Gilbert says, intervening sequences are both frozen remnants of history and the sites of future evolution.

Who's primitive, bacteria or animals?

At first scientists suggested that the intervening segments arose more than a billion years ago when higher organisms diverged from bacteria and blue-green algae. It seemed reasonable that intervening sequences were acquired as part of the increased genetic complexity required for progressive evolution of elaborate higher life forms. So far, the interruptions have been found only in higher organisms (eucaryotes) and not in bacteria and blue-green algae. When scientists introduce copies of a natural animal gene into a bacterium, the bacterium is unable to process a gene containing silent sequences, according to a report in the June 14 NATURE.

Crick suggests that the nuclear membrane is the key to the distribution of split genes. Animal and plant cells have nuclei with membranes; bacteria and blue-green algae do not. In addition, he says that intervening sequences have been found in animal viruses that send their genetic message into a cell's nucleus, but not in viruses that remain outside the nucleus in the cytoplasm.

The presence of intervening sequences in higher organisms, but not in bacteria, can also fit an alternative model. Instead of assuming the eucaryotes gained intervening sequences, the predecessors of bacteria may have lost theirs. W. Ford Doolittle of Dalhousie University in Nova Scotia has argued that the eucaryotic genome with its intervening sequence is the primitive form. It is easy to imagine an organism losing pieces of non-essential DNA, eliminating excess baggage during evolution. But it is more difficult to envision how sequences of DNA could insert into the middle of a gene without completely disrupting critical functions. Because some scientists have suggested that intervening sequences accelerate evolutionary change, Doolittle suggests that in streamlining their genetic machinery to its efficient modern form, bacteria lost their potential for evolution into complex organisms.

Silent regions may promote evolution

A role for intervening sequences in facilitating evolution was proposed soon after the sequences were discovered (SN: 10/1/77, p. 214). At a recent symposium at Wesleyan University, Gilbert reviewed the ideas and evidence that have amassed for intervening sequences as sites of future evolution.

Part of the evidence for an evolutionary role is negative — the silent sequences don't seem to be doing anything else. Gilbert says that when genes of evolutionarily distinct animals are compared, there are generally more differences in the intervening regions (which Gilbert calls "introns") than in the regions that direct protein production. "This suggests they are not doing something specific," Gilbert says.

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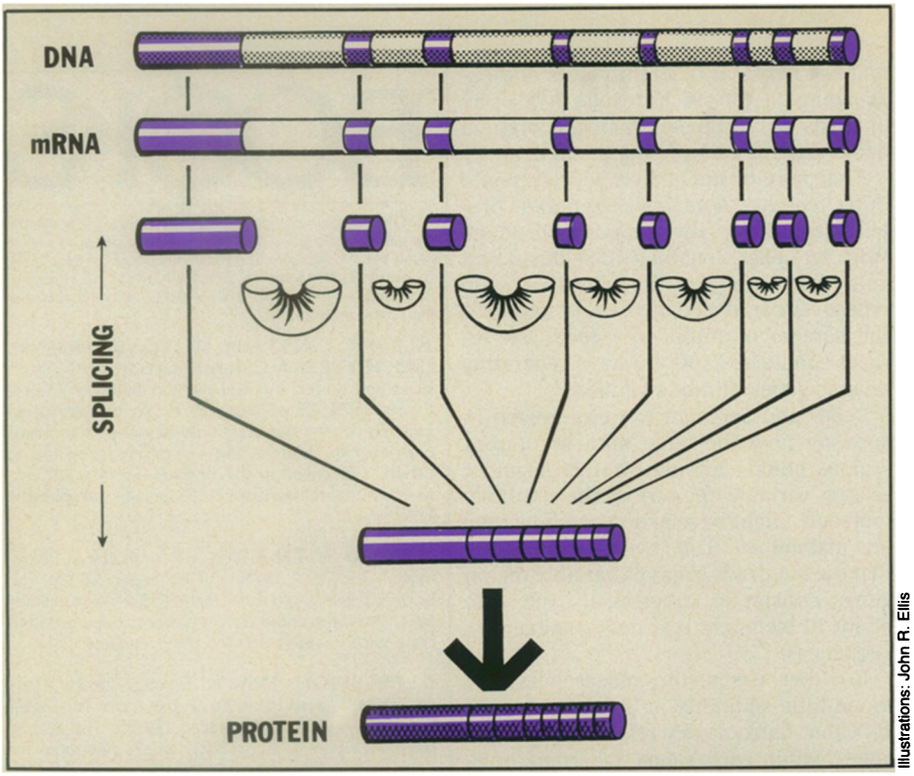

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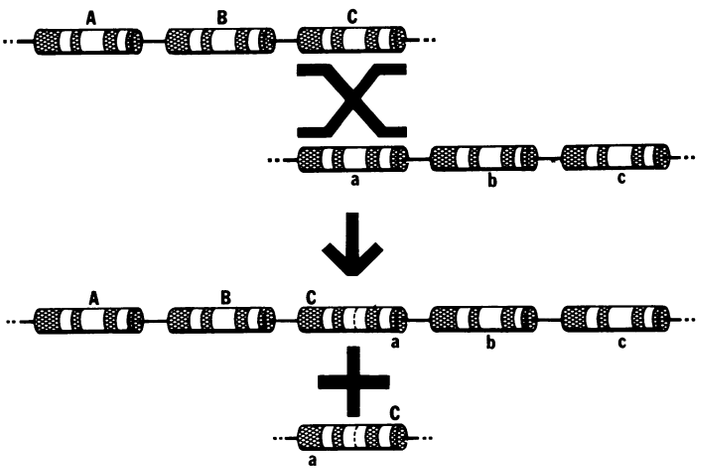
Genes are often mosaics of coding (colored) and silent (white) regions. Enzymes snip the silent regions out of messenger RNA *before it directs protein synthesis.*



Similarities in the silent regions can make them inviting places for breakage and resealing between two chromosomes. When such "crossover" is uneven, the number of genes on each chromosome can rapidly change.

Gilbert has gained confidence in his evolutionary argument from a recent analysis of rat genes. A rat makes two slightly different varieties of insulin, each the product of its own gene. About half the insulin made by a single rat is of each type.

Gilbert and collaborators recently examined these two insulin genes. They found two intervening sequences in one of them, but only one intervening sequence in the other. Because both genes clearly are functional, Gilbert argues, the silent sequence is not essential to the individual animal. The researchers are now analyzing insulin genes from other animals to attempt to determine whether the rat's second intervening sequence is an old element held in common with other animals or a new element recently added to the gene.

Intervening sequences, and the mechanism of splicing segments out of messenger RNA, may be the solution to classical problems of evolutionary genetics. How can small changes at points along a chromosome produce major changes in a gene's product? And how can major changes come about to make a new gene without sacrificing the genes already present?

More genes for evolution

The new view of genes as mosaics of coding and silent regions helps explain how some simple mutations could initiate a dramatic change. A mutation at the boundary of a region to be cut out of the messenger RNA may alter the "snip here" signal. For instance, if the mutation renders the signal unrecognizable to the snipping enzymes, an entirely new sequence of amino acids might be added to the original protein.

In another scenario, a mutation in the signal might allow the signal to be recognized, but less efficiently than before. A cell's enzymes then might make the appropriate cut only some of the time. The result would be simultaneous production of both the original product and a new gene product.

Gilbert speculates that, under such conditions, evolution could seek new solutions without destroying the old. A new protein could arise without sacrificing an old function. If the new product was advantageous to the organism, natural selection would provide pressure for its gene to be duplicated. Then the new product could be made in larger amounts. Gilbert says that a previous hypothesis, that a gene must be duplicated in the chromosome before it is dramatically altered, is unsatisfactory. There is no clear selective advantage in duplicating a gene before it is altered to make a new protein.

Spreading the gene out along the chromosomes, an obvious result of intervening sequences, also is likely to have evolutionary significance. Such an arrangement would increase the probability of shuffling portions of a gene. Thus, regions of different genes or beneficial mutations in different parts of the same gene could get together more easily.

One shuffling model proposes that a new intervening sequence is produced by moving a section of coding DNA along with parts of the silent sequence on either side of it to a new location in the chromosome. The DNA might insert into an existing intervening sequence to produce two silent regions and one coding region where there was only a silent region previously. The translocated coding region might end up within a gene for a different protein. Thus, when a messenger RNA is made, and the intervening segments cut out, a new sequence of amino acids appears within an old protein. The advantage of this hypothetical evolution is that the added amino acid segment already had evolved in its old location to fold up neatly and to perform some function.

Gene structure — protein function

Several investigations of complex proteins and their genes support the idea that a gene segment corresponds to a functional region of its protein.

For example, an antibody molecule has a "heavy" chain and a "light" chain. Each chain has two regions — one that is constant among all antibodies of the family, another that is variable and contains the amino acids that give the molecule its ability to bind a specific chemical. Susumu Tonegawa of the Basel Institute for Immunology in Switzerland and Gilbert find that the gene for each chain includes intervening sequences. In each case there is a silent region between the DNA coding for the constant region and that coding for the variable region. Other intervening sequences break the gene at points corresponding to structurally distinct portions

of the final protein, Gilbert says.

Another example is the globin protein that combines with heme to become hemoglobin, which carries oxygen in animal blood. Gilbert says that the protein has three structural segments — the central piece that contacts the heme and two wing portions. He suggests that the central piece represents a primitive "miniglobin." The wings may have been added for greater efficiency during later evolution.

The association of functional and structural areas of a protein with separated segments of its gene excites molecular biologists. It makes the DNA sequence appear to be more than a linear sequence of arbitrary code. "It's a delightful outcome. At the DNA level, structural and functional aspects are written out," Gilbert says. He anticipates that biologists in the future will be able to infer the underlying functional units of proteins from the structure of their genes.

Restacking the deck

A different mechanism often considered in gene "shuffling" is more like cutting a deck of cards. When the chromosomes of a cell pair up before cell division, homologous DNA strands frequently break and the strands from the two chromosomes join. This phenomenon, called "crossover" (SN: 7/8/78, p. 27), is analogous to two identically arranged decks of cards being cut in the same spot and the top portions exchanged. The result is that the genes at one end of the chromosome are linked to new associates at the other end. Generally, the more distance there is between points on the chromosome, the more likely it is that a crossover event will recombine them. By analogy, if the queen of hearts and the ten of diamonds have 42 cards between them in the pack, it is far more likely that a cut of the deck will separate them than if one was on top of the other.

To extend the analogy even further, if a fistful of jokers were inserted in each deck between the cards of interest, the probability of cutting the cards so as to separate the cards of interest would again increase. Thus Gilbert says that inserting unrelated DNA sequences between parts of the gene coding for different portions of a protein increases the ability of crossover to recombine those pieces of DNA. If, for instance, two individuals had beneficial mutations in different portions of the gene for one protein, in the offspring those mutations are most likely to recombine into one chromosome if they are spaced far apart on the DNA.

Crossover does not always involve breakage at exactly the same point on two chromosomes. Sometimes the DNA molecules pair out of alignment, especially when regions of the DNA that are not completely homologous have similar sequences. In those cases crossover creates one molecule with extra DNA and another missing a stretch. In the card analogy, cutting two decks at different places and exchanging the top stacks results in one pile of cards being thicker than the original decks and the other being thinner.

That sort of unequal crossover could bring together domains of separate proteins to make a new composite product. Work by Leder, Jonathan G. Seidman and colleagues supports the idea that such crossover could also increase or decrease the number of copies of a gene. The increase could be another way of generating diverse genes during evolution.

Leder and Seidman were interested in knowing how the large number of DNA regions coding for one protein segment (kappa variable region) of the antibody molecule might have arisen and how they are maintained. The investigators found that the hundreds of kappa variable region genes consist of subgroups, each with about 10 members that have similar DNA sequences.

In other cases, the researchers had found little similarity in the sequences of DNA that flank closely related genes. The genes within each kappa subgroup, however, are flanked with similar intervening sequences. These flanking regions with their similarity should be inviting sites for unequal crossover, Leder says. Because the unequal crossover can dramatically vary the number of genes, limiting recombination to single subgroups would protect against catastrophic loss of all the kappa coding regions. The most that would be lost in a "mini-catastrophe" would be a single subgroup.

This evolutionary model can be tested, Leder reasons. He predicts that closely related species of animals would differ occasionally in the number of genes within one of the subgroups. To test this hypothesis, Leder looked at eight species of Asian wild mice collected by Robert Callahan. All the mice have similar numbers of genes in one subgroup, but one species almost totally lacks another subgroup. This result, Leder says, supports the possibility that coding DNA can be lost or gained during evolution through unequal crossover.

It is difficult to argue about the evolution that has already occurred, because scientists can never know all the past pressures that shaped natural selection, Leder says. The investigators need instead to make models that generate testable predictions. For instance, the evolutionary origins of antibodies, which are found even in simplest vertebrates but not in lower animals, may be unraveled by looking for functionally different proteins that contain similar DNA sequences.

Using the details of the contemporary DNA to answer questions about the evolutionary past is the most satisfactory procedure now available. "We can't think of an experiment that will allow us to evolve the immune system again," Leder says, adding jokingly, "with the time and money available." □

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