Jumping Genes: A Common Occurrence in Cells

Traditionally, genetic structures have been viewed as stable entities. A gene at a specific site, for example, would not be expected to move elsewhere or to lose its function unless it mutates or undergoes recombination. Since spontaneous mutations occur in bacteria at frequencies of about 10^{-6} and recombinations at frequencies of about 10^{-4}, these events do not, for all practical purposes, alter genetic structures in individual cells.

Evidence that contradicts the view of stable genetic structures has, however, been accumulating. Within the past 2 years it has become clear that, in bacteria, genes and segments of DNA move onto and off of chromosomes and move from place to place on chromosomes with frequencies as great as 10^{-2}. Moreover, molecular biologists are becoming convinced that similar phenomena occur in chromosomes of higher organisms. This new picture of "jumping genes" is elucidating previously unexplained effects in bacterial genetics and, in addition, is providing clues to the resolution of problems in the evolution and developmental biology of higher organisms.

A number of investigators have found that various segments of bacterial DNA move freely from place to place on bacterial chromosomes and among bacterial chromosomes, viruses, and plasmids (small pieces of extrachromosomal DNA that replicate independently and can be directly transmitted from bacterium to bacterium). These movements were detected by two independent means. First, movements of short segments of DNA, about the size of genes, were noticed because they turn off the expression of whole blocks of genes expressed subsequent to, and controlled along with, the gene at the site to which they move. (This effect was first noticed in 1968, but its full significance was not appreciated until much later.) Four specific DNA sequences ranging in size from about 700 to 1400 base pairs have so far been characterized in this way. They are termed insertion elements and denoted IS1, IS2, IS3, and IS4. Second, within the last 2 years, larger segments of DNA that contain genes which confer resistance to antibiotics were observed to move. For example, R. Hedges and A. Jacob of the Postgraduate Medical School in London showed that pieces of DNA containing an ampicillin resistance element can jump from plasmid to plasmid. David Botstein, Nancy Kleckner, and their associates at the Massachusetts Institute of Technology showed that DNA containing a tetracycline resistance gene can move from plasmid to virus to bacterial DNA. Although these segments, called antibiotic resistance elements, also turn off gene expression, their movements can most easily be detected by monitoring the presence or absence of antibiotic resistance genes.

Antibiotic resistance elements resemble insertion elements in several respects. Not only do both classes of movable DNA segments turn off gene expression, but both seem to select sites to which they will move in a nonrandom way. The degree of this specificity is not completely characterized for either insertion elements or antibiotic resistance elements.

Some antibiotic resistance elements are structurally related to insertion elements. At both ends of several resistance elements are identical copies of insertion elements; these copies are thought to be necessary for the movements of the resistance elements. Those antibiotic resistance elements that do not include copies of insertion elements at either end include instead repeated sequences of DNA whose relation to insertion elements is unclear, but which also seem necessary for movement.

Roles of Moving DNA

As the movements of insertion elements and antibiotic resistance elements were discovered, the roles of these DNA segments in the lives of bacteria began to be elucidated. Norman Davidson and his associates at the California Institute of Technology found that insertion elements are crucial to the process of chromosomal gene transfer during bacterial mating. An *Escherichia coli* cell can transfer its DNA to another *E. coli* cell when a specific plasmid, the fertility, or F, plasmid, integrates itself into the donor bacterium's chromosome. Davidson and his associates discovered that the known insertion elements are present in several copies on *E. coli* chromosomes and that the F plasmids contain the insertion element IS2. The F plasmid recognizes IS2 sequences on the *E. coli* chromosome and integrates into the chromosome at the site of an IS2 sequence. Thus the IS2 sequence represents the site where the F plasmid and bacterial chromosome interact.

The repeated sequences at the ends of antibiotic resistance elements also seem to define the regions where these elements interact with plasmids, viruses, and bacterial chromosomes. On plasmids, for example, these repeated sequences are found at the boundary between regions of plasmid DNA necessary for replication and regions conferring antibiotic resistance. Thus many molecular biologists are proposing that insertion sequences or repeated sequences at the ends of antibiotic resistance elements constitute what have been described as "joints for the modular construction of chromosomes." They believe that regions bounded by such sequences could replicate independently of other gene sequences, and that this may lead to the spread of antibiotic resistance genes when bacteria are grown in the presence of antibiotics either in culture or in the wild.

The antibiotic resistance elements enable bacteria to become resistant to antibiotics and therefore to enhance their pathogenicity. These elements are transferred from bacterium to bacterium by way of plasmids and viruses. Fred Heffron, Stanley Falkow, and their associates at the University of Washington studied about 35 naturally occurring plasmids carrying genes for resistance to ampicillin. These plasmids constituted a diverse group, differing in size, composition, and species specificity. Yet all of these plasmids had in common a segment 3×10^6 daltons in length, which contained identical ampicillin resistance genes.

Falkow and his associates believe that antibiotic resistance elements can hop from benign plasmids to plasmids carrying genes that code for toxins. Thus bacteria that pick up plasmids carrying such genes would pick up antibiotic resistance genes as well. In addition, L. P. Elwell, Falkow, and their associates postulate that the movement of antibiotic resistance elements between unrelated plasmids can account for the observation that type b *Haemophilus influenzae*, which can cause meningitis and other infections, suddenly acquired genes for resistance to ampicillin and tetracycline. The ampicillin resistance genes in this

bacterium, at least, are identical to those studied previously in *E. coli* and other enteric organisms.

Because insertion elements and antibiotic resistance elements turn off the expression of blocks of genes when they integrate into DNA, many investigators believe that this event may play some normal role in bacterial metabolism. They point out that bacteria contain several copies of each known insertion element and these elements can and do move about (since viruses infecting bacteria are known to pick up these elements). There is also some evidence that insertion elements and antibiotic resistance elements may turn on as well as turn off gene expression, thus enabling these segments of DNA to act as regulatory switches.

The first report that insertion elements may turn on genes was published about a year ago by H. Saedler, now at the Universität Freiburg, together with H.-J. Reif of the Universität Köln, S. Hu of the California Institute of Technology, and Davidson. These investigators found that IS2 turns on genes in *E. coli* when this insertion element is oriented in the opposite direction to that in which it turns genes off. Now Heffron and Falkow find that the ampicillin resistance element prevents gene expression only when it is in a particular orientation, whereas in the other orientation, it mutates the gene in which it is inserted but does not turn off, and may even turn on, the expression of adjacent genes. Sankar Adhya of the National Cancer Institute has found that when IS1 is prevented from turning off gene expression, this insertion element turns on the expression of adjacent genes.

As interest in the effects of moving bacterial DNA grows, molecular biologists are discovering and, in some cases, rediscovering, evidence that similar phenomena may occur in higher organisms as well. The evidence is of two types: structural and genetic. While many questions about the interpretation of these results remain open, the analogies to moving bacterial DNA are striking.

The structural evidence consists of similarities between the orientations of various sequences of eukaryotic DNA and the orientations of sequences on either end of antibiotic resistance elements of bacteria. When double-stranded pieces of eukaryotic DNA are separated, the single-stranded pieces form numerous stem and loop structures that resemble structures formed by bacterial DNA containing antibiotic resistance elements. The stem of such a structure is formed when two complementary DNA sequences separated by a nonrepeated DNA sequence pair up. The loop is formed from the sequence between the two complementary sequences. Many regions that can form stem and loop structures exist in DNA from eukaryotic cells. For example, Robert Baker and Charles Thomas of Harvard Medical School estimate that the *Drosophila* genome contains approximately six copies of each of about 5×10^3 such regions. These regions, they report, constitute 2 percent of *Drosophila* DNA.

Stem and loop structures formed in bacteria from antibiotic resistance elements arise because the identical end sequences of these elements are oriented in opposite directions (that is, a specific sequence occurs at one end of the element and its complement, oriented in reverse order, occurs at the other end). For example, Kay Ptashne of the California Institute of Technology and Stanley Cohen of Stanford University Medical School reported that IS3 brackets the interior segments of the tetracycline resistance element in this way. Falkow and Heffron found that a short sequence, not related to any known insertion element, is oriented in opposite directions at either end of the ampicillin resistance element. Douglas Berg and Julian Davies at the University of Wisconsin, together with Bernard Allet and Jean-David Rochaix of the University of Geneva, discovered that a long sequence, not related to any known insertion element, brackets the interior of the kanamycin resistance element in opposite orientations.

Genetic Evidence of Movement

Genetic studies of certain eukaryotes have yielded evidence that DNA segments may move around, although whether these moving segments resemble moving bacterial DNA remains to be determined. Those who study *Drosophila* genetics have known for at least 10 years that chromosomal segments sometimes undergo unusual deletions and rearrangements. For example, M. M. Green of the University of California at Davis noticed that deletions frequently occur in the white eye gene region of the *Drosophila* genome. He points out that deletions induced by mutagens or that are due to spontaneous mutations remain unchanged indefinitely. The deletions he studied in the white eye region, however, often are very unstable and frequently are followed by still larger deletions.

Green and others have reported that, in addition to undergoing deletion, *Drosophila* genes are often capable of moving. Green saw two different transpositions of genes from the X chromosome of *Drosophila* to chromosome 3. To complete the analogy to moving bacterial DNA, Green, together with B. Rasmuson and Britt-Marie Karlsson of the University of Umêa in Sweden, saw mutations in *Drosophila* that turn off gene expression in somewhat the way insertion elements turn off gene expression in bacteria. The *Drosophila* genes will suddenly cease to be expressed but can spontaneously regain their functions as if a DNA segment had inserted itself, shut off gene expression, and subsequently popped out of that site on the *Drosophila* genome.

Some of the best evidence that DNA segments move about in higher organisms comes from corn genetics studies begun in the late 1940's by Barbara McClintock of Cold Spring Harbor Laboratory in New York. McClintock says that the world wasn't ready for her results when she started this work but that now there is a surge of interest in this aspect of the genetics of corn.

McClintock noticed that moving segments of DNA, which she called "controlling elements," occur sporadically in corn and can be detected because they change the expression of various genes. A controlling element, for example, may turn off the activity of a gene by becoming integrated within, or close to, that gene. The controlling element may subsequently leave that site, restoring the activity of the gene, and may become reintegrated elsewhere. In addition, controlling elements may turn on gene activity and may bring the expression of nearby genes under the regulation of the controlling element.

The effects of controlling elements on gene expression in corn and the increasing evidence that bacterial insertion elements and antibiotic resistance elements can turn on as well as turn off gene activity are prompting many molecular biologists to propose that moving segments of DNA may function in the development of cells and their transformation by viruses. Such DNA segments could provide means to shut off gene activity in differentiated cells of higher organisms. Similarly, they could be used to stimulate the expression of genes transcribed only under special circumstances; and they may be related to the mechanisms whereby viruses transform normal cells into tumor cells. The DNA's of several viruses that integrate into chromosomes of higher organisms have been shown to contain at one end short sequences that

are repeated in the opposite orientation at the other end. If these bracketing sequences cause the viruses to act like bacterial insertion elements or antibiotic resistance elements, they could turn host genes on or off.

Another implication of the preliminary evidence that DNA of higher organisms might move about is to provide a solution to a problem posed by those who study evolution. These biologists have long maintained that gene rearrangements, duplications, and deletions are of primary importance in evolution and that point mutations alone cannot explain the rapidity with which organisms evolved (*Science*, 8 August 1975). Movements of segments of DNA by mechanisms like those that cause bacterial DNA segments to move could permit such changes in gene organization.

The apparent universality of moving DNA segments and their potential importance has caused a great deal of interest in the mechanism that causes DNA to move in bacteria. Many questions are still open, but molecular biologists believe they have made progress in understanding how and why bacterial DNA moves and exerts its effects and what model systems may prove useful in the future. They now have some idea why insertion elements turn genes off and how antibiotic resistance elements integrate into DNA.

Adhya, together with Donald Court, Asis Das, and their associates at the National Cancer Institute propose that insertion elements contain a sequence recognized by a protein, called rho, that terminates gene transcription. Gene transcription then cannot start again until another initiation signal is encountered. These initiation signals occur at the beginnings of blocks of genes controlled in concert. Thus if an insertion element is incorporated into a gene, transcription would stop in the interior of that gene and no subsequent genes controlled along with the gene mutated by the insertion element would be expressed.

In support of their proposition, Adhya and his colleagues find that IS2 contains a site recognized by rho and that bacteria whose rho gene is mutated do not have gene transcription stopped by any known insertion element. Moreover, a gene product of the bacterial viruses lambda and P22 prevents rho from acting and prevents insertion elements from turning off gene expression.

Although the question of how insertion elements and antibiotic resistance elements integrate into DNA is still open, recent evidence provides some indication of what DNA sequences may be necessary for integration.

Heffron and his associates report results supporting the idea that the inverted sequences at the ends of an antibiotic resistance element are recognized by an enzyme coded by that element. The enzyme then would join up the end sequences with bacterial, virus, or plasmid DNA sequences and catalyze the insertion of the antibiotic resistance element.

Consistent with this hypothesis, Heffron and his colleagues find that three areas of the ampicillin resistance element are necessary for the element to insert itself into DNA: namely, the two end sequences and a segment in the middle of the element. If either of the two end sequences are deleted, the element cannot insert under any circumstances. If the middle segment is deleted, the element can insert only if another ampicillin resistance element containing that segment is present in the same cell. This indicates that the end sequences have a structural role in insertion and the middle segment contains a gene whose product is necessary for insertion.

Viruses Are Models

Many investigators believe that one way to study the integration of insertion elements and antibiotic resistance elements is to study the integration of certain bacterial viruses such as lambda and mu into bacterial DNA. Lambda normally inserts only in a specific site on the *E. coli* chromosome; but when that site is deleted, it integrates at a lower frequency at numerous other sites. In this respect it resembles the insertion elements which have some, but not absolute, site specificity. The virus mu, on the other hand, inserts itself seemingly at random and with a high frequency into bacterial chromosomes. Mu cannot reproduce unless it integrates into host chromosomes, indicating to many that mu insertions may proceed in ways analogous to those of insertion elements and antibiotic resistance elements.

One reason for the interest in lambda as a model for integration is that lambda codes for its own enzyme that catalyzes its integration into host DNA. Moreover, L. Enquist and his associates at the National Institute of Child Health and Human Development report that mutations altering the integration enzyme of lambda alter the places where lambda inserts into the *E. coli* chromosome. Howard Nash and his colleagues at the National Institute of Mental Health have devised an in vitro system to study the mechanism with which lambda inserts in vivo. They and others expect to use this method to gain a detailed understanding of the biochemistry of this event.

Some investigators question the relation between the integration of lambda and insertion elements because lambda DNA forms circles before it integrates into bacterial chromosomes. Thus far, there is no evidence that insertion elements or antibiotic resistance elements form circles. On the other hand, present evidence indicates that mu DNA apparently does not form circles prior to its integration into bacterial chromosomes; hence, some researchers believe that the mu system may be a better model than the lambda system for studying the integration of insertion elements and antibiotic resistance elements.

The analogy between the excision of mu and that of insertion elements and antibiotic resistance elements has, until recently, been unsatisfying. Mu normally takes some bacterial DNA with it when it leaves host chromosomes. Both insertion elements and antibiotic resistance elements, however, may move from host chromosomes without taking along bacterial DNA, although they more often do leave behind deletions of bacterial DNA when they excise. Now, however, A. I. Bukhari of Cold Spring Harbor Laboratory has isolated mutants of mu that mimic the excision of insertion elements and antibiotic resistance elements by excising either with or without host DNA. These mutants were formed when IS1 integrated into the genes of mu. This indicates that information contained in the DNA sequences of insertion elements may play a role in determining how these elements excise and, specifically, may permit them to excise without carrying along fragments of bacterial DNA. Thus mu, which is easier to obtain and work with than insertion elements or antibiotic resistance elements, may provide a means to study both insertion and excision of these elements.

Now that the initial reluctance of researchers to accept the idea of moving DNA has been overcome, the study of bacterial insertion elements and antibiotic resistance elements has caught the fancy of molecular biologists. Questions abound, but bacteria, plasmids, and bacterial viruses are particularly amenable to study. This means, many believe, that it may soon be possible to explain how jumping genes jump and what the jumps imply.—GINA BARI KOLATA

Additional Reading

1. A. I. Bukhari, J. Shapiro, S. Adhya, Eds., *DNA Insertion Elements, Plasmids, and Episomes* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., in press).