A Movable Feast in the Eukaryotic Genome

Nucleated cells carry movable elements very similar to those of bacteria. The elements may–or may not–have an evolutionary role

During the past few years most discoveries about the genes of nucleated cells have emphasized how they differ from the genes of the much simpler bacterial cells. The identification of noncoding DNA sequences in eukaryotic, but not in bacterial, genes is a prime example. But there are also some strong similarities, and one of these, it is now becoming clear, is the presence in the eukaryotic genome of movable DNA sequences that closely resemble those found in bacteria.

The suggestion that these might exist in the higher species is not new, since it dates back to work done, beginning in the late 1940's, by Barbara McClintock of Cold Spring Harbor Laboratory. McClintock's conclusion met, for the most part, with disbelief until a few years ago when insertion elements were discovered in bacteria (*Science*, 30 July 1976, p. 392). The evidence for their presence in eukaryotes has been mostly genetic, however, and it has only been within the past 2 to 3 years that investigators have been able to get their hands on the eukaryotic elements and determine their structures. What they are finding is that movable elements, no matter where they originate, have the same structural organization. Melvin Green, who is at the University of California at Davis, says, "They are everywhere, in bacteria, yeast, *Drosophila*, and plants. Perhaps even in mice and men."

What the movable elements are doing there is still something of a mystery. Because they can cause mutations and gene rearrangements, there have been suggestions that the elements may serve as generators of variability, the raw material of evolution. They are also known to turn genes on and off and may help to control gene expression. But these suggestions are by no means universally accepted; alternate proposals suggest that movable elements may not necessarily contribute to an organism's fitness or its evolutionary adaptability, that they are, in fact, a type of "parasitic DNA."

The current story on the eukaryotic elements began about 5 years ago in the laboratory of David Hogness at Stanford University School of Medicine. Hogness, with David Finnegan, Gerald Rubin, and Michael Young, identified and characterized a class of fruit fly (*Drosophila melanogaster*) genes that are present in many copies, perhaps 30 or so in each cell. Repeated gene sequences are a common feature of the eukaryotic genome, but the ones identified by the Stanford group were considered somewhat unusual because they are dispersed throughout the genome, not linked together in tandem arrays as are most other repeated genes.

The best studied of the repeated genes is named *copia* to reflect the fact that copious amounts of RNA copies of the gene are present in the cell in the RNA fraction (polyadenylated RNA) that contains the messenger molecules which direct protein synthesis. But work from Hogness's and several other laboratories have made it clear that there are additional families of dispersed repeated genes in the fruit fly, and these have been given more prosaic number designations. The total is uncertain, but Rubin, who is now at the Carnegie Institution of Washington, and Young, who has moved to Rockefeller University, have evidence suggesting that there may be a large number of *copia*-like elements—as many as 20 or 30 families—in the *Drosophila* genome. As much as 5 percent of fruit fly DNA may be composed of the elements.

Investigators think that the elements move from one chromosomal location to another because mapping studies, done by Rubin and Young among others, have shown great variability in the chromosomal locations of the elements in different *Drosophila* strains. They are even found in different locations in different individuals of the same strain. Ordinarily, patterns of gene distribution in individuals of the same species are relatively constant. Young says, "The dispersed repeated sequences seem to be nomadic. They occupy no fixed position, but wander from place to place in the genome."

Although there is little similarity between the nucleotide sequences of members of the different families of movable elements, the ones that have been thoroughly studied thus far all have the same structural organization. The elements range in size from 5000 to 7500 base pairs. The sequence of bases at one end of each molecule is a direct repeat of the sequence at the other end, with these direct repeats running 300 to 500 base pairs in length. The Rubin group, which has determined portions of the nucleotide sequences of *copia* and element 297, has shown that there is a short inverted repeat of a few base pairs at each end of the direct repeats.

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Exactly the same features are found in the movable elements of bacteria. Moreover, according to Ronald Davis of Stanford University School of Medicine, they are also found in the elements of another eukaryotic organism, yeast, which is evolutionarily unrelated to *Drosophila*.

Another point of resemblance between the insertion elements of these very different species is their mode of insertion into the genome. Studies of the bacterial elements show that they generate a direct repeat of a short sequence of bases in the cellular DNA when they integrate. The same is true for the elements of yeast and *Drosophila*.

Other aspects of the elements' mode of insertion remain mysterious. Gerald Fink of Cornell University explains, "We know that they do get in and out, but we don't know how they do it. It is clearly not by homologous recombination." Homologous recombination, which happens normally in cells and produces exchange of genes between

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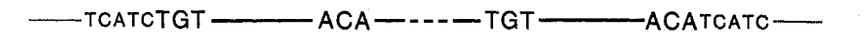

Diagram of copia inserted in cellular DNA

Insertion of the copia *element (sequence shown in large type) generates a duplication of five base pairs of cellular DNA, here TCATC. The ends of the direct repeats of copia are bounded by the inverse repeats TCT . . . ACA. In the complementary chain, which is not shown, the sequence would be ACA . . . TCT.*

chromosomes, is also the mechanism by which some kinds of DNA segments insert into cellular DNA. But homologous recombination requires that the DNA segments recognize, and bind to, regions of cellular DNA with similar base sequences. The movable elements described here cannot be integrating in this way because there is little or no resemblance between their sequences and those of the chromosomal DNA.

In yeast, however, copies of individual direct repeats, without the remainder of the insertion element, are often found scattered throughout the genome. They may provide sites of homology for element insertion, although this is not a requirement; Fink has shown that the elements can go in at sites where there is no sequence resemblance.

As yet there is no direct evidence for insertion elements in higher eukaryotes, such as birds and mammals, but these species are infected by viruses that appear to be descendants of the elements. Some 10 years ago, Howard Temin of the University of Wisconsin School of Medicine suggested that retroviruses, which can cause cancerous tumors in laboratory animals, evolved from movable genetic elements in cells. These viruses have RNA as their genetic material, and their duplication requires that the RNA be first copied into DNA by an enzyme called reverse transcriptase. Since this is the reverse of what had been thought to be the normal flow of information from DNA to RNA, the RNA tumor viruses became known as retroviruses. The DNA transcripts (called proviruses) can either direct the synthesis of additional copies of viral RNA or be incorporated into cellular DNA, where they may trigger malignant changes.

Influenced by this history, Temin made his suggestion, which is now being borne out by structural studies done by Kunitada Shimotohno in his laboratory and also by other investigators. "The structures of the proviruses," Temin says, "are the same as those of the bacterial and other insertion elements." In addition, when proviruses integrate they, too, cause duplication of a small segment of the host cell DNA, and there is no resemblance between the provirus and cellular DNA sequences.

Temin and Shimotohno propose that two small movable elements, the precursors of the direct repeats, may have inserted themselves around a gene coding for an enzyme that copies DNA. This would be the ancestor of the viral reverse transcriptase. Such an element might move about the genome directly as do other movable elements. Or it might first be transcribed into RNA, which could be copied into a DNA provirus, which could then insert itself back into the genome at another site. Eventually a virus might be formed if the element acquired the additional information needed to package the RNA transcript so that it could move between cells.

The role of the movable elements themselves is currently a topic attracting considerable interest, as well as its share of controversy, as investigators try to figure out what they do. The elements may not carry structural information needed to make cell components. The bacterial elements may carry antibiotic resistance genes, but this ability might be a recently acquired adaptation to the widespread use of antibiotics; it may not reflect any primary function of the elements.

The eukaryotic elements are not known to carry genes coding for any specific characteristic, even though they direct the synthesis of large quantities of RNA transcripts. According to Rubin, these RNA's are translated in the test tube to produce several polypeptides, but as yet no function can be attributed to the proteins.

There are indications that the elements are not absolutely necessary for life. Young finds that *Drosophila simulans*, a species of fly closely related to *D. melanogaster*, has very few, if any, of the elements. Still, *D. simulans* occupies more or less the same habitats as does *D. melanogaster*. In competition experiments between the two species, which were carried out in Young's laboratory, neither seemed to have any particular edge over the other. Young concludes. "It is difficult to imagine that the sequences carry any crucial information for making a fly."

Most investigators have focused on the possibility that movable elements may instead help to control gene expression. The bacterial and maize elements are noted for their ability to turn genes on and off as they move about their respective genomes. In addition, they promote chromosomal rearrangements, including deletions and inversions, thus giving rise to mutations. Gene rearrangements, such as those that occur during the development of antibody-producing cells, are being recognized as a normal feature of eukaryotic development. Speculations that movable elements are responsible for developmental rearrangements are intriguing but, for now at least, they are just that, speculations.

As generators of mutations, the elements may also have evolutionary significance. And there is a large body of evidence showing that the elements, both those of bacteria and those of yeast and fruit flies, do generate mutations.

Fink and his colleagues have analyzed two spontaneously occurring mutations of the yeast *his4* locus, which codes for one of the enzymes needed to synthesize the amino acid histidine. They find that one of these mutations is caused by insertion of a movable element very similar to the one described by Davis. The other is also caused by an insertion element, which differs from the first by the replacement of about two-thirds of the base pairs.

The two mutations studied by the Fink group are highly unstable, often reverting to the wild type. Instability is a prime characteristic of mutations caused by insertion elements because they can move out of a given location just as readily as they move in. The Cornell workers have shown that this is the case for the *his4* mutations. The reversions are caused by excision of the insertion element, which is usually imprecise, leaving behind a portion of the inserted segment or taking out a piece of cellular DNA.

Unstable mutations in the fruit fly have also been linked to movable elements. For example, Green studied certain unstable mutations in eye color and found that their properties closely resemble those of bacterial mutations known to be caused by insertion elements. Another eye color mutation, this one characterized by G. Ising and his colleagues at the University of Lund, Sweden, has been mapped to at least three different chromosomal locations, a finding that suggests that the mutant gene moves. Recently, Walter Gehring and Renato Paro of the University of Basel obtained evidence indicating that the mutant gene is closely linked to a *copia* element. They proposed that the gene's

changes in location are mediated by the element.

Insertion of movable elements can also disrupt the normal course of development in the fruit fly, according to Welcome Bender, who originally worked with Hogness, but who is now at Harvard Medical School. He has shown that two spontaneous mutations, which cause severe abnormalities in the development of the segmented *Drosophila* body, are caused by the insertion of fairly long DNA segments containing about 10,000 base pairs each. A reversion of one of the mutations is accompanied by the loss of the insert. Benders says, "We see striking developmental effects of apparently simply mutations."

The hybrid offspring of certain matings of wild-type fruit flies with laboratory strains are characterized by a complex of abnormalities, including frequent and unstable mutations, chromosomal rearrangements, and sterility. This mating incompatibility is called hybrid dysgenesis, and it, too, appears to involve the activities of movable elements. For example, one type of hybrid dysgenesis occurs when males of one strain, designated P, mate with females of another strain, designated M. Production of the abnormalities in the hybrids requires the interaction of a chromosomal factor contributed by the males with a cytoplasmic factor contributed by the females. According to William Engels of the University of Wisconsin, the P factor has all the attributes of a movable element, and the abnormalities seen are consistent with this being the case.

Just because movable elements have been firmly implicated as generators of mutations, however, it does not necessarily follow that they are important for evolution. In fact, some investigators, including W. Ford Doolittle and Carmen Sapienza of Dalhousie University in Halifax, Nova Scotia, and Lesley Orgel and Francis Crick of the Salk Institute, have proposed that movable elements may have no such function at all*. The idea is that DNA segments, which are present in the genome in multiple copies and which furthermore can move about, will be just about impossible to lose. Provided their presence does not impose too high a drain on the cell's energy production, the cells bearing them will survive, and the DNA segments will be perpetuated as parasitic DNA molecules.

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*See *Nature*, 17 April 1980, p. 601; 26 June 1980, p. 617; and 18/25 December, p. 645.

Fingers of Salt Help Mix the Sea

Once ridiculed as imaginary, tiny salt fingers are helping to explain how extremes of temperature and salinity mix in the sea

Sandy Williams was becoming just a bit discouraged. Here, in the Atlantic beyond Gibraltar, was the fourth part of the world that he had searched, as yet with no success. If he was ever going to find salt fingers, the layers of fingerlike, interwoven protrusions of water predicted by some theorists, this should be the place.

Twelve hundred meters below his ship's deck, a huge tongue of warm, salty water, which had slipped out of the Straits of Gibraltar and down into the depths of the Atlantic, was mixing with the cooler, less salty water of the Atlantic. Those conditions seemed ideal for salt fingering, the special kind of mixing in which large bodies of water are supposed to mingle through centimeter-wide, vertical columns of flowing water. Williams, from the Woods Hole Oceanographic Institution, had looked in other likely places but found nothing to convince his colleagues that he had seen salt fingers.

As Williams rolled the wet, freshly developed film exposed by his deep-diving camera package onto a reel, the chief scientist of the cruise, a chemist, happened by and asked "Where are these salt fingers of yours?" Without having inspected a single frame, Williams grabbed a handful of wet film from the tank, held it up, and said "Right there." To the surprise of both of them, the shimmering images of salt fingers were there, as clear as they ever would be seen.

With those observations in 1973, salt fingering, initially only a theoretical and laboratory curiosity, took a big step toward being regarded as a significant mechanism for oceanic mixing. Impressed by these and other observations, oceanographers now accept salt fingering as a common, efficient mixing process in polar waters where water masses having different temperatures and salt contents meet to form fronts, much as warm and cold air masses collide to form weather fronts. Still controversial is salt fingering's role in determining the distribution of heat and salt over large areas of the tropical and subtropical oceans. Several recent studies suggest to some researchers that salt fingers help to mix these warm, salty surface waters with the cooler, fresher waters beneath them. Such mixing would be of particular interest because salt fingers, which are driven by molecular processes, have the unique ability to transport salt more efficiently than heat.

The molecular nature of salt fingering was a major obstacle to its acceptance as a possible mixing process. According to David Evans of the University of Rhode Island and others, many physical oceanographers clung to the philosophy that such interactions between molecules could not affect a body of water tens or even thousands of kilometers across. The ocean, they said, is simply too turbulent to allow it. Considering that kind of resistance, it is not surprising that salt fingers were for some time viewed as a mere theoretical oddity. Their history is usually traced back to a thought experiment presented in 1956 by Henry Stommel, Duncan Blanchard, and Arnold Arons in which they envisioned a "perpetual salt fountain" formed by sticking an aluminum pipe down into the sea. The pipe walls would allow heat but not salt to pass into the pipe. Once "the pump" was primed, seawater would be driven up the pipe by the differences in the distribution with depth of temperature and salinity.

In 1960, Melvin Stern of the University of Rhode Island pointed out that a pipe was not necessary to separate the heat and salt—nature can discriminate between heat and salt on the basis of the speed with which they diffuse through seawater. The 100-fold higher rate of molecular diffusion of heat over that of salt

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