JUMPING GENES

Barbara McClintock's
Scientific Legacy
JUMPING GENES:
Barbara McClintock’s Scientific Legacy

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Barbara McClintock was born in 1902. She was, from a young age, a nonconformist, and decided early on to study science—something few girls did at the time. She enrolled at Cornell University’s agriculture school in 1918 and emerged in 1927 with a Ph.D. in botany. While at Cornell, though barred from the plant breeding program (because she was female), she became a respected member of the flourishing maize genetics community, developing a reputation as a geneticist of unparalleled ability.

Despite her renown, McClintock faced many obstacles as her career progressed. Opportunities for advancement were limited for women scientists, and McClintock did not find a “home” laboratory until she was nearly forty years old. She arrived at the Carnegie Institution’s Department of Genetics at Cold Spring Harbor, New York, in 1942. It turned out to be the ideal place for her to grow her corn. Unencumbered by teaching or administrative duties, she was able to devote full time to the experiments that would lead her to her famous discovery of transposition, or “jumping genes.”

Although Barbara McClintock died in 1992, the legacy of her discovery lives on. Hundreds of scientists now study transposable elements on the molecular level. To them, and to many others, McClintock remains one of the greatest scientists of the twentieth century.
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... she shook a few shoulders and more than a few minds, engendering the courage to break free and see the unfamiliar with new eyes, rearrange the pieces in novel ways, remaining faithful only to what is really there, not the dogma of the day.

Nina Fedoroff and David Botstein
The Dynamic Genome: Barbara McClintock’s Ideas in the Century of Genetics, 1992

Thirty years ago, scientists envisioned an organism’s genome—its total array of genes, the same in each cell—as a neat and orderly place, a sort of gentlemen’s poker parlor. Variations in genetic material from individual to individual were thought to occur only as a result of simple changes in single genes.

Since then, a whole host of “illegitimate” genetic recombination processes—serving to rearrange the genes in dramatic ways—have been uncovered. Genomes are now seen more as riproaring gambling halls than quiet clubs.

At the top of the list is a phenomenon called transposition. In transposition, movable DNA segments called transposable elements, or “jumping genes,” move from one location to another on a chromosome, or to a different chromosome. When these small pieces of DNA move, they have the capacity to cause simple mutations or to promote gross chromosomal damage.

Transposition was discovered in Indian corn, or maize, by Barbara McClintock during the 1940s. More than two decades would pass before movable DNA elements would be discovered in bacteria, and several more years before they would be found in animals and other plants. In 1983, at the age of 81, Barbara McClintock received a Nobel Prize, and the world took notice.

But McClintock, a person of intense privacy, was not interested in fame. In fact, when she first heard the news that she had won a Nobel,
she went out to pick walnuts. Later, she acknowledged her debt to the organism that had yielded its secrets to her. "It might seem unfair," she said, "to reward a person for having so much pleasure over the years, asking the maize plant to solve specific problems and then watching its responses."

To the public, the romance of the "corn lady's" life—a woman working alone and largely unrecognized until finally winning science's most prestigious prize—gave her a heroic dimension. But to McClintock's scientific colleagues, the Nobel was just the culmination of a lifetime of achievement. To them, McClintock was, indeed, a legend, but not because of her solitariness or unconventionality. Rather it was her spirited intelligence, intense concentration, and remarkable passion for science. She discovered that genes could move before the advent of molecular genetics, before scientists even knew what the gene was made of. Using only her eyes, her wits, and her microscope, she analyzed a series of genetic crosses to arrive at a conclusion that was so far ahead of its time as to border on the heretical.

McClintock made her discovery at Cold Spring Harbor, New York, at the Carnegie Institution's Department of Genetics. McClintock was a member of the Department from 1942 until her retirement in 1967. She then became a distinguished service member of the Carnegie Institution, and she remained so—still active in her lab at what became the Cold Spring Harbor Laboratory—until she died in 1992.

At Carnegie, the study of transposable elements continues in the molecular research of Nina Fedoroff, Allan Spradling, and Maxine Singer. This essay is about these present-day scientists. But throughout, it is also indirectly about Barbara McClintock, who provided the rich genetic tapestry into which the molecular discoveries are woven, and whose remarkable insights still have the power to amaze, stimulate, and provoke.
McClintock’s Heresy: Genes Are Not Fixed

Yellow, green; wrinkled, smooth; short, tall; colored, white. . . . These are some of the characteristics in the garden pea that led the monk Gregor Mendel to a revolutionary concept of heredity in the 1860s. After years of breeding pea plants in the garden of his European monastery, Mendel concluded that physical traits in peas were passed from parent to offspring by some kind of heritable “elements.” Dismissed by his contemporaries as simplistic, Mendel’s laws of inheritance received little attention until after his death. At the turn of the century, when the laws were rediscovered, scientists confirmed them, showing that Mendel’s elements, later renamed genes, might reside on the chromosomes in every cell.

For the first half of the twentieth century, the science of genetics flourished. Using primarily fruit flies and corn, the early geneticists bred organisms in controlled crosses, asking how specific characteristics are passed on from one generation to the next. They created “maps” showing roughly where specific genes were positioned along the chromosomes. Virtually everyone believed that those positions were fixed, much like the rooms of a house are fixed, and that an organism’s genome was a stable, orderly place.

Enter Barbara McClintock. In 1951, at a symposium at Cold Spring Harbor, McClintock announced her finding that genes could move. She had painstakingly accumulated evidence for this phenomenon over many years, crossing different strains of corn plants with one another in a frenzy of midsummer activity, and then patiently analyzing the results.

McClintock had earlier discovered that chromosomes in certain stressed maize cells broke and re-fused with other broken chromosomes and that a cycle of re-breaking and re-fusing continued throughout many cell divisions. (A cell, she found, will not tolerate broken chromosomes; a broken end quickly fuses with another.) In some plants undergoing the trauma of this breakage cycle, McClintock found, submicroscopic pieces of chromosomes could, during cell division, detach from their original positions and emplace elsewhere.

Though McClintock could never actually see the moving chromosomal segments, she learned how to “read” the colors and patterns they created in the kernels and leaves by their movement into and out of genes. If, for example, a segment moved into a gene that coded for purple pigment, it could turn the gene off and the
kernel would be colorless. If, during subsequent cell divisions, the segment moved out of the gene in some cells, the gene in those cells would resume function and the resulting kernel would have sectors of purple spots (see figure below). McClintock also learned how to relate such outward manifestations of transposable element movement to chromosome damage seen in her microscope.

McClintock discovered and analyzed two “families” of transposable elements in maize. Each had a complete element that could move by itself, and a defective element that could move only in the presence of the complete element. She called the first family the Activator-Dissociator, or Ac-Ds, family. It was this family that she discussed in 1951 at Cold Spring Harbor. Although her discussion of how she found Ac and Ds was somewhat complicated, the message—that both elements could move—was clear.

The reaction of those present was puzzlement, in some cases hostility. McClintock was saying that the genome was dynamic. It was like being told your kitchen could hop into the attic. It was just too bizarre. Furthermore, in her unconventional interpretation of the results, McClintock was venturing into even less familiar territory. She believed that movable elements were part of a control system in maize that operated to regulate the timing of gene action. Indeed, she called the elements “controlling elements.” It appeared to her that somehow severe chromosome breakage stimulated them to move when they weren’t expected to. And when they did, they affected genes not previously under their regulatory control.

In the years following her 1951 announcement, McClintock tried several times to tell her story. Only a handful of people were interested. She soon tired of trying. For many years, she published only

Above: A gene that encodes a pigment in the kernel’s aleurone layer remains functional (a) until Ds moves into it, turning it off (b). In the presence of an Ac, Ds moves out of the gene in some cells, giving rise to a spotted kernel (c). Right: Maize kernel with Ds insertions. (Figure adapted from “Transposable Elements in Maize,” by Nina Fedoroff, ©1984 by Scientific American, Inc.)
Barbara McClintock's discovery of transposable elements was not pursued on the molecular level in corn until the late 1970s, when Nina Fedoroff of the Carnegie Institution's Department of Embryology in Baltimore, Maryland, began an adventure that occupies her to this day. Above, during a visit to the Department in 1985, McClintock spoke with Fedoroff and members of her lab.

in the pages of the Carnegie Institution Year Books. It wasn’t until the late 1960s, by which time a new era of molecular genetics was at hand, that transposition—as well as other kinds of genome rearrangements—were discovered in bacteria and yeast. With the help of recombinant DNA and genetic engineering techniques, scientists soon found transposable elements in virtually every plant and animal, including humans, in which they looked.

Despite the ubiquity of movable genetic elements, they remain among the most puzzling aspects of biology. These bits of DNA come in a bewildering array of sizes and behaviors. They are interspersed randomly throughout an organism’s genome, often in great numbers and in many different families. The elements generally move only during mitosis (when cells divide), but no one fully knows why or how often they do so. Though they may be stimulated to move by various external disturbances or stresses (such as x-ray treatment or poison), transposable elements may be capable of spontaneous movement in unstressed cells.

Transpositions can be enormously destructive. Not only can the elements alter individual genes, they can also rearrange an entire genome, at the same time promoting massive chromosomal deletions. Why, then, do transposable elements exist at all? Why do their host genomes tolerate them?

No one knows. Basically, they are a mystery. To Nina Fedoroff, who has studied transposable elements in maize for fifteen years, they can be understood only as part of the maize plant’s developmental fabric, possessed of its own unique history, within whose constraints they have survived over time.
On the Molecular Trail

Nina Fedoroff first met Barbara McClintock in 1978. Fedoroff was then a fledgling scientist—a postdoctoral fellow at Carnegie Institution’s Department of Embryology in Baltimore, Maryland. She had come to Cold Spring Harbor to give a talk about her research. McClintock missed the lecture and later apologized, inviting Fedoroff into her lab for conversation. Fedoroff was impressed by the older scientist’s wide-ranging and lucid discourse.

When Fedoroff returned to Baltimore, she began reading in the old Carnegie Year Books where McClintock had published her experimental data. It was, she later said, the most remarkable learning experience of her life. “Like a detective novel, I couldn’t put it down.”

Soon thereafter, Fedoroff—newly appointed a staff member at the Department—decided to abandon the organism she was then studying (the frog) and begin growing corn. She planted her first crop with seeds given her by McClintock in the summer of 1979.

When the planting season was done, Fedoroff and her colleagues—the postdocs, graduate students, and technicians who then shared her lab—set out to explore the results using the molecular tools of the modern biochemist.

Fedoroff is fully immersed in the molecular age. But her objective required that she learn the classical methods of maize genetics. She accomplished this, she says, by suffering. “I didn’t read McClintock’s writings twice. I read them over and over and over again. It’s probably the hardest thing I’ve ever done, becoming a maize geneticist.”

But her efforts paid off. McClintock’s genetic data has guided her well. “The molecular has enriched the genetic, and the genetic has enriched the molecular,” Fedoroff says. “It just pulls you on and on and on.”

The first transposable element Fedoroff and her colleagues isolated was an Ac element. At the time, no one had yet isolated a transposable element from a higher organism. Indeed, it took Fedoroff and her colleagues four years of
painstaking lab work before Ac was firmly in hand.

Their Ac was, they found, a short segment of DNA consisting of 4,563 nucleotides, about as long as an average gene. It had a short sequence of eleven nucleotides at one end that appeared at the opposite end in reverse, or mirror-image, order. Except for this “inverted repeat” (a characteristic now known to be common to a large class of transposable elements), there seemed nothing unusual about the element.

Before long, the Fedoroff group—and other labs around the world by this time on the same trail—had in hand several more Ac elements and a number of Ds elements. While all the Ac elements were nearly identical in nucleotide sequence, the Ds elements were not. Some were only a couple of hundred nucleotides long, while others were nearly as long as Ac (see figure below). Some contained sequences not found in Ac; others resembled Ac only at their ends. But all appeared to be close relatives of Ac. Indeed, all needed to be in the presence of Ac in order to jump: This is what made Ac and Ds an element family.

One of the Ds elements they examined was identical to Ac except it lacked a short sequence (194 nucleotides long) that was present in Ac.

![Diagram of Ac and Ds elements](image)

**Diagrams of an Ac (top) and three Ds elements show sequences missing from the Ds elements as blank spaces. The elements share nearly identical terminal repeats. (Sequences are shown as abbreviations of the nucleotides adenine, thymine, cytosine, and guanine.) The smallest Ds element at bottom has no sequences in common with the Ac element except for its terminal repeat; its sequences are thus represented by the small open boxes.**

Fedoroff, a modern molecular biologist, had to learn the older language and methods of corn genetics. She also had to learn how to grow corn. It was hard work. Each plant had to be tagged and watched carefully. When the plants were ready to be fertilized, great care had to be taken in order to prevent promiscuity. Paper bags were thus placed over the tassels and ears (above) early in development so that the plants did not mate randomly. Today, while she continues to study corn DNA, Fedoroff no longer grows corn. “We’ve exhausted what classical genetics can do,” she says.
Because the lack of this short sequence served to immobilize the element, Fedoroff concluded (and was later proved correct) that the missing sequence was part of a gene that encoded a critical enzyme necessary for movement. To this day it is not known exactly how this enzyme, called transposase, promotes transposition, but it appears to do so by cutting the DNA at the element’s inverted repeat ends, thus releasing the element from its site on the chromosome. The end sequences can then attach to DNA cut apart at another location, on the same or a different chromosome. Ds elements cannot move on their own because their transposase genes are either missing or incomplete. These elements thus appear to be nothing more than defective copies of Ac, perhaps crippled in the process of transposing.

Watching Ac work

Once the nucleotide sequence of the Ac element was known, Fedoroff and her colleagues set about developing an experimental “assay” system where they could “watch” the element at work. The tobacco plant proved to be ideal. A maize transposable element, they learned, could be put into a tobacco cell by first splicing it into a bacterial plasmid (called Agrobacterium tumefaciens Ti plasmid) that normally infects plants.* Once inside the tobacco cell, the Ti plasmid (and its Ac passenger) inserts into a tobacco chromosome.

To determine whether Ac could work in its new environment (i.e., promote transposition), Fedoroff added a second Ti plasmid to the tobacco cell. The second plasmid carried a detection system—a gene that codes for an enzyme that can turn a colorless substance in the plant blue. Smack into the middle of the “blue” gene, however, Fedoroff inserted a Ds element. The blue gene was therefore turned off.

If Ac was working, Fedoroff reasoned, it should be able to move Ds out of the blue gene. (Ds, remember, cannot move on its own.) This is exactly what happened. The transposase enzyme made by Ac

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* A plasmid is a small DNA molecule inside bacterial cells now widely used in recombinant DNA work to ferry genetic material between cells.
promoted the transposition of Ds out of the blue gene, allowing the
blue gene to resume normal function. The blue spots appearing on the
tobacco leaves thus provided telling evidence that Ac did indeed work
in tobacco.

The tobacco assay system was to prove invaluable to Fedoroff and
her colleagues, not only for studying elements in the Ac-Ds family, but
for studying elements in another family of maize transposable
elements—the Spm family.

Tackling Spm

Barbara McClintock discovered the Suppressor-mutator, or Spm,
system a decade after she uncovered the Ac-Ds system. The Spm family
is genetically and functionally more complex than the Ac-Ds family,
but the basic components are similar. It contains complete elements
that can move by themselves (Spmn) and defective derivatives (dSpmn)
that can move only in the presence of the complete element.

Nina Fedoroff now concentrates most of her energies on the Spm
system. She is keenly interested in unraveling on the molecular level a
peculiar phenomenon that McClintock observed: the ability of an Spm
element to suddenly become silent—unable to transpose—for no
apparent reason. McClintock determined that an inactive Spm was not
the same as a dSpm. For, unlike a dSpm, a silent Spm
may become independently active again. A dSpm, in
contrast, remains crippled.

McClintock found that an inactive element could
be reactivated in the presence of an active element. To
Fedoroff, this suggested the intriguing possibility that
Spm encoded a protein (or proteins) that could
somehow “talk to” an inactive Spm, turning it back on.

Counting Genes

In studying how Spm elements communicate,
Fedoroff has inadvertently found herself in the
middle of a new and increasingly popular field of
study—the inactivation of plant genes. Scientists have
been aware for many years that genes may become
inactive when introduced into a plant genome as
multiple copies. Explains Fedoroff: “People introduce
many extra copies of a gene into a plant to get high
expression of some compound. All of a sudden, the
trait becomes unstable and the genes unexpectedly
turn off.” What it seems to mean, she says, is that a

Maize kernels where the Spm
element is inactive (deeply
pigmented), active (colorless), and
undergoing reactivation (speckled).
plant knows exactly what its genetic constitution is. "It's as if the plant had a way of detecting extra gene copies and turning such genes off."

The maize plant appears to be no exception. Spm elements, like many transposable elements, have the ability to multiply themselves during chromosome replication. An element can multiply, for example, by transposing from a site that has replicated into one that has not. When replication is complete, one of the chromosomes will have two elements while the other will have one (see figure, below). Fedoroff suggests that the maize plant recognizes the extra elements as extra genes and turns them off. It does so by attaching methyl groups (CH₃) to certain regions of their DNAs. Methylation is a fairly common gene-silencing mechanism in both plants and animals.

Revolutionaries of the Genome

Spm elements, however, have evolved a way to rid themselves of methyl groups and turn themselves back on, thus circumventing the plant's accounting system. They make a protein—called TnpA—that can bind to the exact same DNA sequences in Spm where the methyl groups bind. Fedoroff has analyzed this protein in great detail. She has found that it plays not one but several roles in the control of Spm activity. Still, she does not yet know exactly how the protein is able to reactivate a methylated element.

What she has found is that reactivation by TnpA is a relatively rare event. It appears, she says, that most silenced elements "come out to play" only in stressful situations, for example, when the chromosomes are subjected to trauma, as in McClintock's initial experiments.

During chromosome replication, when an Ac transposes from a chromosome site that has already been replicated to one that has not (upper diagram), the transposed element will replicate again when its new site replicates. One of the two daughter chromosomes will contain two Ac's and the other (lower diagram) will have one. (Figure adapted from "Transposable Elements in Maize," by Nina Fedoroff, ©1984 by Scientific American, Inc.)
Fedoroff believes, in fact, that inactivation by methylation may be unavoidable for maize transposable elements.

Being silent so much of the time may not be so bad. It may, in fact, be an element’s best chance of survival. When they move, transposable elements tend to lose some of their own DNA. Staying silent keeps them, as Fedoroff says, “from shooting themselves in the foot.” By methylating the elements, the plant also does itself a favor. Active transposable elements can create chaos in the maize genome. A plant with too many active transposable elements, says Fedoroff, can end up “looking more like a cabbage than a corn plant.”

So why, despite their destructiveness, do transposable elements exist in the maize genome? Though she cautions against thinking in terms of “purpose,” Fedoroff speculates that the plant tolerates its transposable elements (she calls them “molecular subversives”) because they might be useful in evolution. Perhaps, she says, the elements are unleashed when the plant is excessively stressed. When they are, they may promote rapid genomic reorganization. In this way, she says, they may act like revolutionaries in human societies. “Like revolutionaries, they tend to surface when things get bad and change is long overdue. And, as in the case of revolutionaries, the outcome of their activity is unknown.”

Fedoroff believes that maize transposable elements have great value for the scientist studying the control of plant genes. “The elements’ genes are subject to the plants’ regulatory mechanisms and can therefore teach us about how plants regulate their genes in development,” she says. But Fedoroff does not believe that the elements themselves will be found to have a role in an individual plant’s development.

One scientist who believes that transposable elements may indeed help regulate development—at least in the fruit fly—works just down the hall from Fedoroff at the Department of Embryology. For years,
Allan Spradling has used transposable elements of the fruit fly as tools to understand processes of development. Now, he is beginning to wonder if those elements might themselves play a critical role in the flies' development.

**A Role in Development?**

The odor of fruit fly culture is unmistakable in Allan Spradling's laboratory. Escaped flies hover above the workbenches, where several individuals—most of them postdoctoral fellows in their twenties, dressed casually in jeans—concentrate on their tasks at hand.

Fruit flies, or Drosophila, are tiny insects that seem to appear spontaneously in the presence of decomposing fruit. Once favored by the early geneticists, the Drosophila genus has become invaluable to molecular researchers who, like Spradling, find in the fly's fast breeding schedule, easy care, and long genetic history an ideal means for studying aspects of gene function in higher organisms.

Spradling has long been committed to the fruit fly—and its transposable elements—in exploring processes of oogenesis, or egg development. Specifically, he uses as his special tool a family of transposable elements called the P-element family. P elements are found in the genome of certain fly strains called, appropriately enough, P strains. Normally, P elements seldom transpose, but when a P-strain male is mated with a female from another Drosophila strain that does not normally contain P elements (an M strain), the P elements

*Allan Spradling at the microscope; cotton-stoppered bottles contain fruit flies.*
start jumping around in the P-M hybrids at very high rates.

Movable genetic elements were found in Drosophila in the early 1970s. Now, more than fifty families are known to exist, comprising from five to ten percent of fruit fly DNA. As in maize, many transposable element families (including P) have both complete and defective varieties, and each complete element contains a gene that encodes a transposase that is necessary for transposable element movement. (Each family of elements encodes its own transposase.)

Ferrying Genes

Spradling first became familiar with P elements in the early 1980s. At the time, a fellow staff member at the Department, Gerald Rubin, had just succeeded in isolating P elements from Drosophila. Spradling wondered if it might be possible to insert a P element into a Drosophila embryo. Would it be expressed in future generations? He began spending a lot of time in Rubin’s lab. The two hoped eventually to develop a way to “harness” a P element, to use it as a sort of ferry in transporting cloned Drosophila genes into Drosophila embryos.

Spradling and Rubin first created, in effect, artificial P-M hybrids, by injecting P elements into M-strain eggs. While many of the hybrids died from the trauma of injection, the offspring of the survivors occasionally contained a single P element. Encouraged, the two researchers then repeated the experiment, but this time they hitched a gene called rosy onto the P element, and inserted both into an egg lacking the rosy gene. (Rosy promotes normal, red eye color. Mutants lacking rosy have brown eyes.) As many as 50% of the survivors produced some offspring with normal, red eyes. And, so too did succeeding generations. Rosy had become a heritable part of the fly’s genome.

Spradling and Rubin were elated. While laboratory gene-transfer methods in higher organisms (such as that Fedoroff developed for maize elements in tobacco) are today commonplace, Spradling and
Rubin's was the first in which introduced genes were correctly expressed. According to one scientist, what they did was to take biology a step beyond cloning. Here, finally, was a way to study working genes directly within an organism.

In an interview many years later, Spradling remembered vividly the feeling their success evoked. "It put a pretty big smile on your face when you went to look at the results of your previous day's experiments. It was one of those situations that you occasionally get doing research where something works much better than you have any right to expect."

The success of their venture was heralded far and wide. In the years immediately following their discovery, Spradling and Rubin received a stream of visitors from all over the world. Many researchers hoped that P elements might be used to transfer genes into other organisms, such as mammals, or at least into economically (or medically) important species of insects (e.g., mosquitoes or houseflies), possibly leading to some mechanisms for pest control.

But this has not yet happened. The real value of the transfer technique remains limited to those researchers like Spradling wishing to study working genes in the fruit fly. Spradling and his colleagues have since used the technique to study how a variety of Drosophila genes function during oogenesis.

P Elements to the Rescue Again

As the years passed, it became increasingly clear to Allan Spradling that there were not just a few genes involved in Drosophila oogenesis, but many hundreds. He was getting frustrated. With standard technology, it took from three to four years to find, isolate, and characterize a single Drosophila gene. "I figured we could spend the rest of our lives finding genes," Spradling said in a 1992 interview, "or we could try and improve the technology."

In 1988, Spradling and his colleagues—this time without Gerry Rubin, who had since moved to California—turned again to transposable elements for help. It was possible at the time to create mutations simply by introducing a few dozen P elements into the genome and allowing them to jump at will, disrupting genes as they did so. But locating those genes wasn't so easy. There were too many P elements hopping haphazardly around, too many mutated genes.

Spradling and two postdoctoral fellows then in his lab, Lynn Cooley and Richard Kelley, found instead that they could make a single mutation in a fly by inducing the transposition of a single P element into a genome lacking any other active P elements. The P element, carrying a marker gene for easy identification, disrupts and
Above: the Carnegie Institution's Department of Embryology sits on a corner of the Johns Hopkins University campus. The Department was founded in 1914 originally for the study of human embryos; later it became home to a breeding monkey colony—one of the first in the world. The Department now houses a veritable zoo of experimental organisms, such as mice, zebrafish, frogs, maize, and Drosophila (right). Using the single-P-element-mutagenesis technique, Spradling and his colleagues have produced a Drosophila gene “library” at the Department that now contains about 700 strains, each containing a single mutation. The library is a valued resource for the Drosophila genetics community worldwide.

...easily identifies the location of a single DNA region, allowing rapid mapping and characterization of the affected gene.

Almost overnight, the task of finding and isolating Drosophila genes was simplified dramatically. Upon publication of the paper in Science explaining the nuts and bolts of the so-called single-P-element mutagenesis process, Spradling once again had the biology world tilting toward Baltimore.

Fixing Broken Chromosome Ends

Allan Spradling is a thoughtful, engaging scientist who received his Ph.D. in molecular biology from MIT. He became a staff member at the Department of Embryology in 1980. In July 1994, he was appointed director. He is also an Investigator of the Howard Hughes Medical Institute. Though the Hughes Institute is based in Bethesda, Maryland, its investigators conduct their research at their parent institutions.

Spradling's enthusiasm for transposable elements remains high. P elements have been an invaluable tool in his laboratory. They have provided a unique window on Drosophila developmental processes, and they have enabled him to study many Drosophila genes of interest.

In recent years, however, Spradling has been doing a lot of thinking about P elements themselves. Though he realizes they are nothing but "strings of nucleotides," he often talks about them as if they are thinking, willful, even "smart" beings. As he talks, he also brings up
Barbara McClintock’s name frequently. It was McClintock, for example, who first observed a phenomenon called chromosome healing, which, in Drosophila, appears to be promoted by transposable elements.

In Drosophila cells, says Spradling, if the end of a chromosome (the telomere) is broken off, the cell soon begins to build another. “It seems to be that at least two different families of transposable elements in Drosophila, HeT and TART, know to go to the ends of the chromosomes,” Spradling says. The elements jump only onto chromosome ends. P elements, and possibly other transposable elements from other families, efficiently transpose just inside the HeT and TART arrays. (See figure on p. 22.) In this way, a new, structurally normal chromosome end may be formed within a few dozen generations. “Before you have time to study it very much,” Spradling says, “it has corrected itself.”

**Rearranging the Heterochromatin**

To Allan Spradling, chromosome healing is interesting because it shows how transposable elements help a genome respond to stress. (In this case, the stress is a broken chromosome end.) But it is in another process—the differentiation of cells—that Spradling believes transposable elements may play an even more important role. Why do some cells become blood cells, for example, and not muscle cells? How transposable elements may promote the turning on of some genes in a cell early in development and the turning off of most other genes in that cell is unclear, but Spradling suspects they may somehow rearrange certain areas of DNA called heterochromatin, and that the new arrangements may affect the cell’s developing function.

Heterochromatin was first recognized decades ago as odd-staining regions of the chromosomes, primarily at the middle and ends. Today, heterochromatin is known to contain copious amounts of repetitive DNA having no known function, as well as a few operational genes. It litters the chromosomes of most higher organisms, accounting for substantial percentages of their genomes.

That cells of higher organisms contain
so much noncoding, repetitive DNA was discovered in mice in 1964 by Roy Britten and colleagues at another of the Carnegie Institution’s departments—the Department of Terrestrial Magnetism in Washington, D.C. Britten and his longtime colleague Eric Davidson proposed that such repeated sequences played an important role in regulating genes, but proof was lacking. Even today, no one knows why organisms contain so much noncoding repetitive DNA. Some regard it as little more than “junk.”

By the early 1970s, it had become clear that the enlarged chromosomes of the Drosophila salivary glands contained heterochromatin that failed to increase during the development of the salivary tissue. In scientific terms, the heterochromatin became “underrepresented.” Other researchers later found that underrepresentation also occurred during the differentiation of many other Drosophila cells containing similar enlarged chromosomes.

Spradling was intrigued. To study underrepresentation more thoroughly, he and his colleagues took advantage of a small, artificially created Drosophila “minichromosome,” which contains two heterochromatin regions surrounding a small, nonheterochromatin region encoding eight genes (see figure, next page). The Dp1187 minichromosome was first made in the 1950s by researchers at the University of California, San Diego, using x-radiation. It is still the smallest known chromosome able to function in a multicelld organism. It can be added to normal Drosophila cells and behave as any other chromosome: its genes are transcribed and it either replicates or it becomes underrepresented in specific tissues during development.

In their experiments using the minichromosome, Spradling and colleagues found that the minichromosome did, indeed, become underrepresented in certain cells. However, it appeared that the reason was not simply a failure to replicate. A more likely explanation was that much of the noncoding heterochromatin was physically excised, or deleted, from the minichromosome at an early stage in tissue development. If true, this would violate one of the dogmas of
One end of the Drosophila minichromosome Dp1187, above, shows two heterochromatin regions (open boxes) surrounding a coding region. The gene for yellow is expressed in a variegated way (sometimes on, sometimes off) depending on the amount of heterochromatin eliminated. (The “hotspot” is where P-family members preferentially jump to rebuild a broken chromosome end, or telomere. Members of the HeT and TART families jump into the subtelomeric heterochromatin region.) A complete Dp1187 is 1,300 kilobases (kb) long. See scale at lower right.

biological science—the theory of DNA constancy. This theory states that every cell of an organism contains the same genes and the same total amount of DNA. But here was an example where the amount of DNA changed in a cell.

Scientists had earlier observed DNA elimination in a few invertebrates, plants, and recently in certain fish. But in studying it, they tended to focus on the eliminated material itself. Spradling, in contrast, is looking at the elimination process. He suggests that certain transposable elements (although probably not P elements) may be programmed to delete certain DNA regions in the heterochromatin in certain tissues during development. “Elimination would alter the DNA sequences that surround the rare heterochromatin genes,” he says. “In this way, the altered sequences might influence the organization and activity of the genes.”

If true, then transposable elements, by interacting with the heterochromatin, may indeed play a role in determining cell function. “This might give us a more complete appreciation of the rest of the genome—not just that part that encodes proteins—in controlling developmental processes,” Spradling says. It would also lend experimental support to a similar (and heretical) suggestion of Barbara McClintock’s.

McClintock believed that the control of genes during development was caused not by alterations to the genes themselves, but by the reorganization of the chromosome (in particular, the heterochromatin) in regions near the genes. It seemed to her that such reorganization was promoted by the action of transposable elements. She wrote in 1952: “. . . one should look first to the conspicuous heterochromatic elements in the chromosome in search of the controlling system associated with initiation of differential genic action . . . ; and secondarily to other such elements, which are believed to be present along the chromosomes and to be either initially or subsequently
involved in the events . . . ." (The "other such elements" are the transposable elements.)

In essence, McClintock was saying the same thing as Allan Spradling. Very few scientists listened to McClintock; her suggestion was as bizarre to them as her notion that genes could jump. But will Spradling's reception be as chilly?

Perhaps not. It has been known for years that a genome can rearrange itself during, for example, the production of antibodies in an animal's immune system. As well, scientists look at chromosomes today not as mere bearers of the genes but as sophisticated systems of control and information processing. And, more and more, they are finding evidence that at least some of the noncoding "junk" on the chromosomes might actually serve a purpose in the genome. Perhaps the time is ripe to reassess the role of heterochromatin and jumping genes.

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**Movable Elements in Humans**

One scientist with an open mind on this question is Maxine Singer. Singer says that Spradling is "one of the broadest thinkers in biology today." Singer is herself fascinated by transposable elements. When her schedule allows, she explores movable elements in human DNA at her laboratory at the National Institutes of Health. But Maxine Singer is also the president of the Carnegie Institution of Washington. As well, she is in constant demand all over the world as one of science's most eloquent and learned spokespersons. Maxine Singer probably spends more time in airports than most people spend in their back yards.

Singer is a long-standing member of the molecular biology community. She was on the organizing committee of the famous 1975 conference in Asilomar, California, for example, where biologists confronted the possibility that serious risks might accompany the then new recombinant DNA experiments. The guidelines these biologists issued were followed rigorously. Yet over the years, most scientists came to realize that the original risk scenarios were overblown. The chance of creating hazardous organisms is today understood to be almost nil.

Despite this, Singer has often found herself on the defensive, arguing in newspaper editorials, at lectures, and even on television that most recombinant DNA research is safe, ethical, and potentially of great benefit to human
society. She provides, for example, a reasoned “no” to the belief that scientists have power to re-create such long-extinct organisms as dinosaurs, or that genetically engineered foods like the new “Flavr-Savr” tomato are dangerous. In an editorial appearing in the August 10, 1993 issue of the Washington Post, for example, she pointed out that the new Flavr-Savr tomato is made in essentially the same way and contains the same ingredients as those “natural” foods we eat every day. She wrote, “... all the foods we eat are the product of previous genetic engineering by cross breeding. Moreover, they have DNA in them, as well as thousands of other chemicals; living things are, in a sense, chemical systems.”

**LINE-1s Emerge**

Maxine Singer is small, intense, and quietly friendly. She speaks slowly and deliberately, keeping a sharp eye on her listener for signs of understanding. Her office at the Carnegie Institution, in downtown Washington, D.C., is handsomely furnished. Photographs of her family and modern artwork cover the walls and tables. One of the pictures she most treasures hangs above her desk. It is a drawing, a caricature really, of Barbara McClintock holding an enormous ear of corn.

McClintock is one of her heroines, Singer says. McClintock was special to Singer long before Singer began her foray into transposable elements. That started accidentally in the mid-1980s. At the time, Singer was running a large laboratory in the National Cancer Institute on the sprawling campus of the National Institutes of Health (NIH) in Bethesda, Maryland. She and her colleagues were studying repeated sequences in monkey DNA. They were investigating the junctures where a kind of noncoding repeated sequence met the coding sequences, or genes. What they
found was something else entirely—a different DNA sequence that was repeated and interspersed throughout the monkey genome in large numbers. They found two kinds of these interspersed sequences: short interspersed sequences (SINES), about 300 nucleotides in length (previously discovered by others), and long interspersed sequences (LINES), up to 6,000 nucleotides long.

At the time, transposable elements had not yet been discovered in mammals. But Singer suspected that monkey LINES just might be transposable. For instance, they appeared to contain a gene that looked a lot like a gene that makes reverse transcriptase. Reverse transcriptase is an enzyme that allows DNA to be "reverse" synthesized from RNA. It enables a class of transposable elements called retrotransposons to transpose.

Unlike other transposable elements, such as those in maize and fruit flies described earlier in this essay, retrotransposons don’t actually move when they transpose. What moves is a copy, synthesized from the retrotransposon’s RNA transcript. In this way, each time a retrotransposon transposes, the original stays put and a new copy is made and inserted elsewhere. (See diagram, above.) In their DNA structure and mode of operation, retrotransposons are very similar to a class of viruses called retroviruses. Retroviruses are responsible for some human diseases, including AIDS and some types of hepatitis. But retrotransposons, unlike retroviruses, are not infectious. They lack the gene that in the retrovirus encodes a special envelope which surrounds the retrovirus and allows it to get out of a cell in a viable form.

About the same time Singer discovered LINES in the monkey genome, scientists elsewhere found similar versions of LINES in the genomes of other mammals, including humans. The human LINES looked much like those in monkeys, and Singer, intrigued by the opportunity to study human biology, decided to study them. She wanted to know by what mechanism the LINES in humans—called
Maxine Singer is wholeheartedly committed to science education—at all levels. In 1989, she founded a Saturday science school at the Carnegie Institution for inner-city elementary school children in Washington, D.C. Above, she helps children with one of their projects.

LINE-1 sequences—transpose, if they did indeed transpose.

Though there may be other LINE elements in humans, LINE-1s are the only ones found to date. They are interspersed randomly throughout the human genome in tens of thousands of copies, composing some 5% of the total DNA. (SINES make up another 5%.)

Because of their great numbers, LINE-1 sequences proved rather easy to find. In time, Singer and colleagues retrieved and sequenced several. Most appeared to be incomplete, that is, they lacked nucleotides found in other LINE-1 sequences. Singer thus suspected that most were inactive in the genome.

Finding cells where LINE-1 sequences might actively transpose proved to be much more of a challenge. To find these LINES, Singer and her group began searching in a variety of cultured human cells for the mRNA that they thought the LINES might transcribe. They knew that the mRNA, like the LINE element itself, would have a distinguishing sequence of adenine-rich nucleotides on one of its ends. (Unlike transposable elements in maize and fruit flies, LINE-1s do not contain inverted repeats at their ends.) Despite this helpful mark, it was only after months of trial and error and what Singer calls a particularly “dogged” search by one of the postdoctoral fellows then in her lab, that they succeeded. They found LINE-1 mRNA only in one type of cell—teratocarcinoma cells. Derived from tumors arising from germ-line cells, these cancerous cells can often be experimentally induced to differentiate, merely by treatment with certain chemicals.

Using the LINE-1 mRNA from teratocarcinoma cells, Singer and her colleagues were able to reconstruct 19 LINE-1 elements that appeared long enough to be functional. Upon sequencing the elements, however, they found that all were slightly (about 1%) different. This was surprising, considering that each was transcribable. Apparently, LINE elements can make mRNA even when they contain slight mutations.

**Catching a LINE-1**

At this time—it was now the late 1980s—Singer and her colleagues had in hand what appeared to be several complete and transcribable
LINE-1 elements. They began experiments on the elements—to learn how they were transcribed, for example, and to learn what proteins they encoded. However, they did not know for sure if any of the LINE-1 elements they were studying could actually transpose.

In 1990, things changed. A group of researchers at the Johns Hopkins University led by the geneticist Haig Kazazian found evidence that a LINE-1 had indeed transposed, inserting right in the middle of a gene—with devastating results. At the time, Kazazian and his colleagues were not looking for LINE-1 elements. They were analyzing the gene that made a blood-clotting protein (called factor VIII) in a large number of hemophiliac boys, searching for ways by which new mutations arise. The factor VIII gene in two of the boys was disrupted by a foreign stretch of DNA that looked suspiciously like a transposable element. Its DNA sequence confirmed that the segment was indeed a LINE-1.

Normally, hemophilia is found in boys whose mothers carry a mutated factor VIII gene on one of their X chromosomes. Because males inherit only one X chromosome, those who inherit a chromosome containing a defective factor VIII gene are inevitably hemophiliac. The factor VIII gene in the mothers of the two boys, however, was normal. The mutation in the sons’ genes had arisen spontaneously, either during maturation of the eggs that gave rise to the two boys, or during the boys’ early development.

When Kazazian and colleagues examined one of the mother’s DNA (using the son’s LINE-1 element as a probe), they found a full-length LINE-1 on her chromosome 22 that matched closely the one in her son’s factor VIII gene. Interestingly, they also found the LINE-1 in the same position on the father’s chromosome 22. It turned out that all primates—humans, chimpanzees, and gorillas alike—have a LINE in the same location. It has apparently been there for at least six million years. The reason why it transposed when it did and where it did—copied from the DNA of the mother or father (it could be from either) to that of the son—is unknown.

Having an apparently active LINE-1 in hand was encouraging to Singer, but experimental proof of activity was still lacking. To this day, no one has been able to get the LINE-1 element to transpose in the laboratory. There’s no lack of trying; Singer and Kazazian (who recently moved to the University of Pennsylvania) have joined forces in this effort, collaborating by phone, mail, and group meetings. LINES appear to transpose so infrequently that the odds of seeing one move are very, very slim.
Maxine Singer is scientist emeritus at the National Institutes of Health, where she maintains a small laboratory in the National Cancer Institute research building. Above, she poses with the postdoctoral fellows in her lab. Left to right: Hiro Hohjoh, Kang Liu, Singer, Julie McMillan, and Andrew Clements. At left: with Clements.

What Does p40 Do?

By the time Singer accepted the presidency of the Carnegie Institution (in 1987), she was too deeply involved in LINES research to imagine giving it up. Besides, she had since high school loved doing science, and had spent most of her career at the bench. Even when her four children were young (they are now grown and pursuing professional careers), Singer continued her research.

Once she joined Carnegie, however, the demands of her presidency and of her increasingly visible status as a national figure have allowed her to spend only about two days a week at NIH. In her absence, the four postdoctoral fellows with whom she shares her lab continue the work, maintaining communication via telephone, e-mail, and faxes.

A big question in the lab involves one of the proteins (in addition to reverse transcriptase) encoded by the LINE-1 element. Singer calls the second protein p40 because it has a molecular weight of 40 kilodaltons. She and her colleagues have cloned the p40 gene and synthesized enough protein to study its basic properties. They also made antibodies against it. Antibodies are natural antagonists designed to find and “lock” into specific proteins, inactivating them. They are useful tools in the molecular biology laboratory because they allow investigators to locate cellular proteins that might otherwise remain hidden.

By introducing p40 antibodies into human cells, Singer and her colleagues were able to find p40—but, again, only in teracarcinoma cells (and in a few other tumor cells). This meant that LINE-1 elements make p40 only in those cells where LINES are transcriptionally (and theoretically transpositionally) active. But what function, if any, does p40 have in the transposition process?

This remains a mystery. The gene that makes p40 occupies a
LINE-1 element diagram shows the p40 and reverse transcriptase genes as open boxes. The shaded areas represent non-coding regions.

position in the LINE-1 element that in retroviruses is occupied by a gene which encodes a protein not even remotely similar to p40. Indeed, the amino acid structure of p40 appears to be different from that of any known protein. (Structure often holds the key to function, says Singer, because certain kinds of structures are associated with certain functions.) Even after a recent exhaustive computer search of all known proteins present in the large human genome data bank, the protein remains unique. “We have very seriously considered the possibility that p40 plays no role at all in transposition,” Singer says. “We can’t say one way or another. Maybe everything needed for transposition is encoded by the other gene. Maybe p40 is something else entirely.”

The “other gene” to which Singer refers is the gene in LINE-1 that makes reverse transcriptase. Singer says that this gene is long enough to make another protein—or proteins. If it does make other proteins, however, it makes them in very small amounts, for Singer and her colleagues have not yet been able to find them.

Another big unsolved mystery about LINE-1 elements involves the mechanism of transcription. Why, Singer wonders, are LINE-1s transcribed almost exclusively in teratocarcinoma cells? “Is it a positive turning on in these cells or a positive turning off in other cell types?”

Even in teratocarcinoma cells, transcription of LINE-1s occurs at very low levels. Furthermore, some factor (or factors) in the intracellular environment appears to suppress translation of the reverse transcriptase gene into protein. (Translation of the p40 gene is also suppressed, but not as much. The two are independently translated.) That translation of the LINE-1 genes is suppressed is hardly surprising, says Singer, considering how destructive the elements can be.

Capturing a Gene

Singer is perched on a chair in her NIH office. Her coffee—never far from hand—is cold. Pieces of paper with scribbles of LINE diagrams litter her desk. She begins to talk about the big picture—to step back from the hands-on science to speculate why LINE-1 elements exist.

She explains that most scientists who study LINES believe the
elements to be little more than parasitic troublemakers. She agrees they are troublemakers. "These elements are destructive; they disrupt genes, and more and more we see in the literature that LINE-1s transpose into genes, as they did into the factor VIII gene." All the same, while she echoes Nina Fedoroff in saying there is no reason to think LINES have a purpose, she remains open-minded. There is something interesting, she says, about the fact that all mammals have retained these sequences, even though they are destructive.

Perhaps, she suggests, LINES exist in humans because, along their evolutionary journey, they "captured" a gene that the organism does need. "If a LINE-1 captured such a gene," she says, "the LINE would have become indispensable. The organism would have to put up with it and all of its circus performing acts." (Or perhaps, she says, it may be that LINES contained their essential gene or genes all along and the organism developed a dependency on that gene.) If LINE-1 elements do indeed contain a necessary gene, she speculates, that gene may be the one that makes p40—whatever p40 turns out to be.

**Conclusion**

That each one of the scientists featured in this essay offers a different perspective about transposable elements is not particularly surprising. Each one brings different attitudes, different techniques, different states of mind to the problem. And, perhaps most critically, each one studies a different organism.

Which one is right? Are transposable elements, as Fedoroff suggests, revolutionaries of the genome? Or do they perform what Spradling suspects are critical roles in the differentiation of cells? Or, do they exist, as Singer speculates, only because they bear essential genes? Perhaps each one of these scientists is right; perhaps transposable elements, exposed to different environments and selection pressures, have assumed a variety of roles in the genomes of different organisms.

Then again, perhaps all are wrong. It is possible that transposable elements exist for no reason other than that they have been successful at replicating themselves. To biologists, this is not difficult to comprehend. Explains Singer: "Life makes a tremendous amount of sense, but only in its own terms, not in human philosophical constructs. In a way, every living thing we see—ourselves included—represents a genome that has succeeded in replicating itself. Though a transposable element's ecological niche is some other
organism’s genome, fundamentally transposable elements are no different from that larger genome. There’s no reason to see a purpose.” Fedoroff, a strong empiricist, agrees. “There is no evidence that any organism cannot get along without its transposable elements,” she says.

All the same, more and more scientists are coming to believe that transposable elements may be important on an evolutionary timescale. They may provide, for example, an important source of genetic variation, or mutations—the raw material of evolution. It was McClintock who first suggested that transposable elements may, through their ability to quickly restructure a genome, allow the genome to respond to new and unexpected events, such as accidental chromosome damage, viral infection, poison, or an altered tissue environment. McClintock even proposed that radical genome restructuring by transposable elements may lead to the formation of new species.

Experiments are not possible on evolutionary time scales encompassing millions of years. But some scientists are exploring the possibility that the gene alterations promoted by transposable elements, usually thought to be either neutral or causing detrimental or lethal effects, may actually give those who carry them a slight edge in natural selection. Some have even suggested that transposable elements might indeed explain rapid bursts of species formation evidenced, for example, during the Cambrian period, 500 million years ago, when many new organisms suddenly appeared in the fossil record.

Meanwhile, only a few, besides Allan Spradling, agree with McClintock’s suggestion that transposable elements also play critical
roles in development. McClintock originally called transposable elements "controlling elements." She came to believe they play a role in the control of gene action during development, perhaps—as Spradling is coming to suspect—indirectly by rearranging a cell's heterochromatin.

More time will be required to learn whether heterochromatin rearrangements are indeed a programmed part of normal developmental patterns. Only with more evidence will we learn whether in this matter, too, Barbara McClintock was far ahead of her time.

Suggested Reading


The Dynamic Genome: Barbara McClintock's Ideas in the Century of Genetics, Nina Fedoroff and David Botstein, eds., Cold Spring Harbor Press, New York, 1992. (A compilation of writings from McClintock's scientific colleagues, telling how they were influenced and inspired by her)


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