

ADDRESS BY PROFESSOR FRED GAGE

RECIPIENT OF THE INTERNATIONAL PRIZE 2011 OF THE FYSSEN FOUNDATION

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Members of the Fyssen Family, Members of the Board of Directors and the Scientific Committee, Ladies and Gentlemen, it is a great honor to be this year's awardee of the Fyssen Foundation's International Prize. I am proud to be in the company of so many distinguished past awardees and colleagues.

I will now give you a brief introduction, description and discussion of the research for which the prize was awarded. I am a neuroscientist and I focus my attention on the brain and the remarkable diversity, adaptability and plasticity that is retained by the brain over the course of a lifetime. Nearly 15 years ago, my colleagues and I discovered that, in a specific structure in the human brain called the hippocampus, there are stem cells that continue to divide and give rise to new neurons throughout life. Subsequently, in the course of studying how these stem cells are regulated and what their unique function could be, we began a search for which genes were expressed uniquely in these cells when the cells make a choice to stop dividing and become neurons. By chance we discovered that many of the genes that are highly expressed in these cells fell into a class of genes called "jumping genes." Such genes, which have been found in mice and humans, can essentially paste copies of themselves into other parts of the genome (the full set of DNA in the nucleus) and alter the functioning of the affected cell, making it behave differently from an otherwise identical cell right next to it. Many such insertions in many different cells would be expected to yield subtle or not-so-subtle differences in cognitive abilities, personality traits and susceptibility to neurological problems.

Our early findings of gene jumping in the brain have led us to another question: given that the brain's proper functioning is essential to survival, why has evolution allowed a process that tinkers with its genetic programming to persist? Although we still do not have a definite answer, increasing evidence suggests that, by inducing variability in brain cells, jumping genes may imbue organisms with the flexibility to adapt quickly to changing circumstances. Therefore, they may have been retained evolutionarily because, from a species-survival point of view, this adaptation benefit has outweighed the risks.

The idea that mobile elements like jumping genes exist and move about in the genome is not new, but evidence that they are so active in the brain came as a surprise. Gene jumping was first discovered in plants, even before James Watson and Francis Crick spelled out the double-helical structure of DNA in 1953. In the 1940s, Barbara McClintock, of Cold Spring Harbor Laboratory, observed that "controlling elements" moved from one place to another in the genetic material of corn plants. She discovered that, under stress, certain regions in the genome could migrate and turn genes on and off in their new

location. The products of McClintock's experiments were now-famous ears of corn with seeds of varying colors—a demonstration of “genetic mosaicism,” in which genes in a particular cell may be switched on or off in a pattern that differs from that of neighboring cells that are otherwise identical.

McClintock's research, which at first encountered skepticism within the scientific community, eventually resulted in a Nobel Prize in 1983. In recent years, it has become clear that the phenomenon of genetic mosaicism is not restricted to plants but also occurs in many organisms, including humans.

McClintock did her work on transposons, which are mobile elements that use a “cut-and-paste” mechanism to move a stretch of DNA from one location to another in the cell's genome. More recent research on mobile elements in the brain has focused on retrotransposons, which employ a “copy-and-paste” approach to insert themselves into new areas of the genome. They essentially replicate themselves, rather than popping out of the surrounding DNA, after which the copy takes up a new position elsewhere.

Retrotransposons make up as much as half of the nucleotides, or DNA building blocks, in the entire human genome. (In contrast, the approximately 25,000 protein-coding genes we possess make up less than 2% of mammalian DNA.) They are descendants of the first primitive molecular replication systems that invaded the genomes of eukaryotes (organisms having cells that contain a nucleus) long ago. Once thought of as nonfunctional “junk DNA,” retrotransposons were first found to be active in human tissues in 1988 by Haig Kazazian's group. In particular, one type of retrotransposon, known as “Long interspersed element 1” (L1), appears to be a key player in the human genome. It is able to hop around frequently probably because it, unlike other mobile elements in humans, encodes its own machinery for spreading copies of itself far and wide in the cellular genome. Analysis of its behavior in cells reveals that, when something prompts an L1 in the nuclear genome to begin the “jumping” process, it first transcribes itself into single-stranded RNA, which then travels from the nucleus to the cytoplasm, where it serves as a template for constructing proteins specified by some parts of the L1 DNA. The proteins then form a molecular complex with the still intact RNA, and the whole complex heads back to the nucleus. There, one of the proteins, an enzyme known as an endonuclease, makes a nick in some part of the DNA. It also uses the RNA as a template for producing a double-stranded DNA copy of the original L1 retrotransposon and inserts this duplicate into the genome where the cut was made. Such “reverse transcription,” from RNA to DNA is familiar to many people today as part of the way that the HIV virus gets a DNA copy of its RNA genome to take up a permanent home in the genome of the cells it infects.

Retrotransposition often fails to run its course, which produces truncated, nonfunctional copies of the original L1 DNA. Sometimes, these snippets or the whole L1 copy have no effect on a protein-coding gene. Other times, though, they can have any of several consequences, both good and bad, for the cell fate. They may, for instance, drop into and thus alter the protein-coding region of a gene. This maneuver can lead to creation of a new variant of the protein

that helps or harms an organism, or this positioning may stop a given protein from being made. In other instances, the newly pasted DNA may fall outside of a coding region but act as a promoter (a switch that can turn on nearby genes) and alter the level of gene expression, i.e., the amount of protein made from the gene, with good or bad consequences for the cell and the organism. When L1 retrotransposons end up in many places in neurons and/or other cells of the brain, the brain will be very different from the one that would have formed without their influence. It stands to reason that such genetic mosaicism would affect behavior, cognition and disease risk and could also help to explain why one identical twin may remain disease-free when a sibling is diagnosed with schizophrenia.

Until recently, most investigators aware of L1 retrotransposition assumed that it mostly took place in germ cells, which make eggs and sperm, or in embryonic stem cells, which give rise to the various cell types in the developing embryo. Yet better detection tools have now revealed that retrotransposons can move around somatic tissues after embryonic development is complete. These events happen more often in the brain than in other tissues, a direct challenge to the longstanding dogma that the genetic makeup of all brain cells in adults is identical and remains stable for the cells' life.

In our laboratory at the Salk Institute for Biological Studies, we monitored gene jumping in a mouse whose cells were genetically engineered to fluoresce green when an L1 element inserted itself somewhere in the genome anywhere in its body. While we observed glowing green cells in many areas of the brain, we also discovered that certain cells in the hippocampus - a region important to memory and attention - glowed green, suggesting that L1s may move around more in the brain than in other somatic tissues. Interestingly, the jumping also occurred in hippocampal neural progenitor cells. In various organs of fully formed organisms, a small population of progenitor cells stands by, ready to divide and give rise to specialized cell types needed to replace cells that die. The hippocampus is one of two regions of the brain where this neurogenesis occurs. Thus in addition to L1s being active during early development when neurons are being born, they can be active in the adult brain in the few areas where new neurons continue to be born into adulthood.

Evidence of active retrotransposition in somatic tissues of humans, and in the brain in particular, came from an analysis of human postmortem material. When comparing the number of L1 elements in brain, heart, and liver tissue, we found that brain tissue contained significantly more L1 elements in each cell nucleus than heart or liver tissue. The difference is a sign that much of the jumping occurred during development, because retrotransposition requires cell division to happen. The counts suggested that each neural cell in humans undergoes an average of 80 L1 integration events, a rate that could well lead to a great deal of variation among cells and in the overall brain activity of different individuals.

We have begun to wonder what might trigger L1 activity. Knowing that the hippocampus is also a site where neurogenesis occurs and that exposure to

novel situations and exercise trigger neurogenesis in mice, we decided to see if exercise might be one spur to gene jumping. We found that, after our transgenic mice ran on a wheel, the number of green fluorescing cells increased about two-fold in the rodents' hippocampuses. Given that novelty and challenge also prompt neurogenesis, we are entertaining the possibility that a new or unfamiliar environment could be another prompt for retrotransposition to occur.

If we are correct and L1 jumping does increase as the nervous system learns and adapts to the outside world, the finding would indicate that each of our brains and the neuronal networks that make them up are constantly changing and are altered by each new experience.

We are continuing to expand the evidence for the hypothesis that jumping genes contribute to human variation in brain processing by moving beyond just counting L1s in DNA. We are attempting to link our data to real events that have either positive or detrimental effects on the lives of real people. In this quest, it is sometimes easiest to pinpoint the bad outcomes that resulted from a gene that jumped, if only because the consequences are so obvious.

In November 2010, our team reported in *Nature* that a mutation in a gene called MeCP2 affected L1 retrotransposition in the brain. Mutations in the MeCP2 gene can induce Rett syndrome, a severe disorder of brain development that almost exclusively affects girls. The discovery that *MeCP2* was mutated in patients with Rett syndrome and other mental disorders raised multiple questions about the molecular and cellular mechanisms of this disease. Our research showed that the mutation in the brains of mice and humans with Rett syndrome showed a significant increase in numbers of L1 insertions in their neurons, a finding that suggests that the jumping genes might account for some of the downstream effects of the *MeCP2* mutation.

L1 activity has also turned up in other disorders. An analysis of the frontal cortex regions of individuals with schizophrenia revealed an increased number of L1 sequences compared to those without the disorder. Circumstantial evidence suggests that L1 elements are an important component of various brain disorders, including autism. Understanding the role of mobile elements in the development of psychiatric diseases might lead to new methods for diagnosis, treatment, and prevention.

The continuing research into jumping genes in the brain could potentially upend an entire academic discipline. Behavioral geneticists often follow groups of identical twins over long periods to control for the effects of genes and determine the environmental contributions to such disorders as schizophrenia. The new findings showing that jumping genes actively revise genomes after an embryo forms overturn the assumption that "identical" twins are genetically alike. Indeed the new discoveries will make it ever harder to disentangle the relative effects of nature and nurture on our psyches.

Many questions remain including why has evolution not destroyed these vestiges of ancient viruses in our cells, given that jumping genes have a high chance of introducing potentially fatal genetic flaws. To answer the question, we should acknowledge that our genomes have always been under attack by viral parasites and other invaders that load our DNA with jumping DNA. The bodies of humans and our evolutionary forebears may not have been able to fully eliminate the interlopers but they have adapted to at least coexist with them by silencing them with a variety of clever mechanisms that mutate and disable them. It also appears that, in some cases, our genomes have commandeered the genetic machinery of L1 retroelements to enhance our own survival, which is one reason that cells may sometimes allow, or even encourage, L1s to jump around the genome under carefully controlled conditions.

One clue to why they persist may come from closer analysis of the finding that mice from a single genetic strain raised under highly controlled conditions vary greatly in their responses to stress and other challenges. The observed behavioral differences are distributed normally in the population (picture a Bell Curve), a pattern that implies that the mechanisms producing this variability are random, as the sites L1 retrotransposon insertions seem to be.

The ability of the L1s to move from place to place in the genome implies that natural selection may, in effect, be rolling the dice in the hope that benefits from helpful insertions will outweigh any deleterious consequences of other insertions.

More possible support for this idea is the discovery that the only lineage of L1 jumping elements currently active in the human genome evolved about 2.7 million years ago, after the evolutionary split from chimpanzees to bipedal humans, a time when our hominid ancestors were first beginning to adopt the use of stone tools. That finding lends credence to the notion that the L1 elements may have helped to build brains that can process information about the environment rapidly and thus more readily meet the challenges of ever-changing environmental and climatic conditions.