

Prova de inglês para ingresso no Mestrado e Doutorado 2015/2

- Prova eliminatória e não-classificatória. Duração: 2h.
 - Nota mínima para aprovação = 5,0.
 - Leia o texto (parcial) de divulgação científica de **Fred H. Gage & Alysson R. Muotri** “*What makes your brain unique*” publicado na Scientific American em Março de 2012 e responda TODAS as questões em português de acordo com o texto.
- 1) Gêmeos idênticos podem apresentar diferenças em seu comportamento. Da mesma forma, ratos geneticamente idênticos tratados da mesma forma em laboratório apresentam diferenças em quais características?
 - 2) Quais fatores podem causar mutações ou afetar o comportamento dos genes? Como?
 - 3) Por qual descoberta a pesquisadora Barbara McClintock recebeu um prêmio Nobel?
 - 4) De que forma o elemento L1 (elemento nuclear intercalado longo) pode “pular” de posição no genoma nuclear?
 - 5) Como um elemento móvel que não se inseriu na região codificante de um gene pode influenciar a expressão do mesmo?
 - 6) De acordo com o texto, qual seria uma possível explicação para o diagnóstico de uma pessoa com esquizofrenia quando seu gêmeo idêntico não apresenta indícios da doença?
 - 7) Quais resultados os pesquisadores do Salk Institute for Biological Studies na Califórnia tomaram como evidência de que os retrotransposons se movem mais frequentemente no cérebro do que outros tecidos somáticos?
 - 8) Experimentos do grupo de pesquisa acima sugerem que exercício físico pode acarretar em uma frequência maior de eventos de transposição pelos elementos L1 por causar neurogênese em camundongos. Qual outro fator também está ligado a neurogênese em camundongos e poderia também acarretar uma maior atividade de L1 no cérebro?

YOUR BRAIN IS SPECIAL.

So is mine. Differences arise at every level of the organ's astonishingly intricate architecture; the human brain contains 100 billion neurons, which come in thousands of types and collectively form an estimate of more than 100 trillion interconnections. These differences, in turn, lead to variances in the ways we think, learn and behave and in our propensity for mental illness.

How does diversity in brain wiring and function arise? Variations in the genes we inherit from our parents can play a role. Yet even identical twins raised by the same parents can differ markedly in their mental functioning, behavioral traits, and risk of mental illness or neurodegenerative disease. In fact, mice bred to be genetically identical that are then handled in exactly the same way in the laboratory display differences in learning ability, fear avoidance and responses to stress even when age, gender and care are held constant. Something more has to be going on.

Certainly the experiences we have in life matter as well; they can, for instance, influence the strength of the connections between particular sets of neurons. But researchers are increasingly finding tantalizing indications that other factors are at work—for instance, processes that mutate genes or affect gene behavior early in an embryo's development or later in life. Such phenomena include alternative splicing, in which a single gene can give rise to two or more different proteins. Proteins carry out most of the operations in cells, and thus which proteins are made in cells will affect the functioning of the tissues those cells compose. Many researchers are also exploring the role of epigenetic changes—DNA modifications that alter gene activity (increasing or decreasing the synthesis of specific proteins) without changing the information in genes.

In the past few years the two of us and our colleagues have come on especially intriguing suspects that seem to operate more in the brain than in other tissues: jumping genes. Such genes, which have been found in virtually all species, including humans, can 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, mounting evidence suggests that by inducing variability in brain cells, jumping genes may imbue organisms with the flexibility to adapt quickly to changing circumstances. Therefore, these jumping genes—or

mobile elements, as they are called—may have been retained evolutionarily because, from the standpoint of promoting survival of the species, this adaptation benefit outweighs the risks.

ANCIENT INVADERS

THE IDEA THAT MOBILE ELEMENTS EXIST and move about in the genome is not new, but the recent 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 the 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 her receiving a Nobel Prize in 1983. In subsequent years it became 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 around the cell's genome. More recent research on mobile elements in the brain had focused on retrotransposons, which employ a copy-and-paste approach to insinuate themselves into new areas of the genome. They basically 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 human genome. In contrast, the approximately 25,000 protein-coding genes we possess make up less than 2 percent of mammalian DNA. The jumping genes are descendants of the first primitive molecular replication systems that invaded the genomes of eukaryotes (organisms having cells that contain a nucleus) long ago. A group led by Haig H. Kazazian, Jr., at the University of Pennsylvania showed in 1988 that retrotransposons, which were once thought of as nonfunctional junk DNA, were active in human tissues.

In particular, one type of retrotransposon, known as a long in-

terspersed 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 called an endonuclease, makes a nick in specific sites in 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.

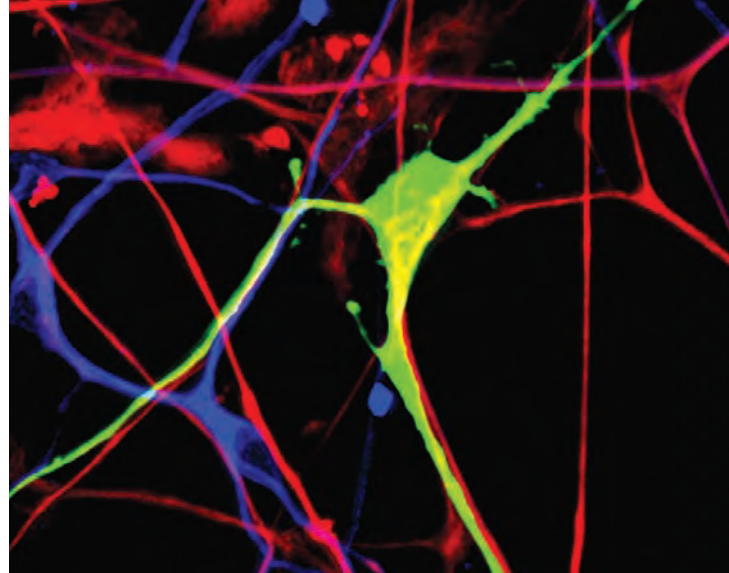
Retrotransposition often fails to run its course, which produces truncated, nonfunctional copies of the original L1 DNA. Sometimes these snippets (or the whole L1 copy) have no effect on a protein-coding gene. Other times, though, they can have any of several consequences, both good and bad, for a cell's 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—the amount of protein made from the gene—with, once again, good or bad results for the cell and the organism. When L1 retrotransposons end up in many places in neurons or in many cells of the brain, or both, the brain will be very different from the one that would have formed without their influence. It stands to reason that such genetic mosaicism could affect behavior, cognition and disease risk and could also help explain why one identical twin may remain disease-free when a sibling is diagnosed with schizophrenia, for example.

WHERE DOES JUMPING OCCUR?

UNTIL RECENTLY, most investigators aware of L1 retrotransposition assumed that it mostly took place in germ cells (ovaries or testes). Although a few clues suggested that L1 genes stay active in somatic tissues (nonsex cells) during early development or later, these clues were generally dismissed. If genes exist merely to propagate themselves, as one evolutionary theory holds, jumping genes would have little cause to remain active in somatic cells because such cells would not pass the DNA to an organism's next generation: after all, the affected cells die when their owner does.

Better detection tools have now revealed that retrotransposons can move around somatic tissues during early development and even later in life. These events happen more often in the brain than in other tissues—a direct challenge to the long-standing dogma that the genetic codes of brain cells in adults are identical to one another and remain stable for the cells' life.

In our lab at the Salk Institute for Biological Studies in La Jolla, Calif., for instance, we monitored gene jumping in a mouse whose



Jumped gene: Nomadic DNA in a neuron gives rise to a protein that glows green after moving to a new spot in the cell nucleus.

cells were genetically engineered to undergo retrotransposition and fluoresce green when an L1 element inserted itself in genomes of a cell anywhere in its body. We observed glowing green cells only in germ cells and in certain brain areas, including the hippocampus (a region important to memory and attention)—which suggests that L1s may move around more in the brain than in other somatic tissues. Interestingly, the jumping was occurring in progenitor cells that give rise to hippocampal neurons.

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 neurogenesis, generation of new nerve cells, occurs. Thus, L1s appear to be active during early development when neurons are being born, but they can also move around in the adult brain in the areas where new neurons continue to be born into adulthood.

Even with the mouse experiments, more evidence was needed that retrotransposition was actually occurring in the brain. We undertook an analysis of human postmortem material that compared the number of L1 elements in brain, heart and liver tissues. We found that the brain tissue contained significantly more L1 elements in each cell nucleus than the heart or liver tissues did.

Much of the jumping had to have occurred during the brain's development because retrotransposition requires cell division—a process that does not take place in the brain, except in two circumscribed areas—to happen after early childhood. An analysis 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.

A recent finding from researchers at the Roslin Institute near Edinburgh and their colleagues supplies further confirmation of L1 activity in the human brain. The researchers reported in 2011 in *Nature* that a total of 7,743 insertions of L1s in the hippocampus and caudate nucleus (which is also involved in memory) in three deceased individuals contained integrated L1 elements. (*Scientific American* is part of Nature Publishing Group.) That study also implied that the emerging portrait of genetic diversity in the brain will only get more complicated as this research moves forward. The Roslin team was surprised to come on about

15,000 members of a class of retrotransposons known as short interspersed elements (SINEs). The preponderant SINE, part of a group known as Alu elements, had never been encountered before in the brain.

Our findings made us wonder what might trigger LI activity. Knowing that the hippocampus is also a site where neurogenesis transpires 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 twofold in the rodents' hippocampus. Given that novelty and challenge also prompt neurogenesis, we are entertaining the possibility that a new or unfamiliar environment could be another instigator of retrotransposition.

If we are correct and LI jumping does increase as the nervous system learns and adapts to the outside world, the finding would indicate that individual brains and the neuronal networks that make them up are constantly changing and alter with each new experience, even in genetically identical twins.