Science & Society

EMBO reports

On jumping genes and other things

Interview with Fred Gage, Adler Professor at the Salk Institute

Nonia Pariente

EMBO Reports (ER): Many thanks for agreeing to talk with EMBO Reports about the fascinating topic of transposable element activity in the human genome. First of all, how did you become interested in this phenomenon?

Fred Gage (FG): I first became aware of transposable elements approximately 14 years ago, when I was studying a gene that modifies fibroblast growth factor (FGF) transcription. This gene turned out to be *MBD1*, which encodes a methyl-binding protein that regulates the transcription of many genes, not just *FGF2*. We knocked *MBD1* out and found some changes in neural progenitor cells. In trying to understand these changes, we realized that the levels of intracisternal A particle (IAP) expression were elevated, a finding that we published in PNAS. That was when I learned about these odd elements and recognized that they were interesting. I also had some background in virology, because we used a lot of retroviruses for introducing genes into dividing cells, so I could see the structures were equivalent and they had some similar properties. But at the time we didn't pursue the issue very far. However, even at that point we hypothesized that the variable behavior that we saw in the *MBD1* knockout animals might be related to this elevated level of IAP expression, but we didn't make a lot of it, and I realized that pursuing the issue was going to be difficult.

ER: What made you decide to pursue the issue further?

FG: At that time, a large part of my lab worked on—and continues today—adult neurogenesis. This is the process whereby stem cells of the adult nervous system give rise to neural progenitor cells (NPCs) that generate neurons in the hippocampus. We developed tools to isolate these stem cells directly from the adult brain, grow them in culture and differentiate them into oligodendrocytes, neurons and astrocytes, as well as NPCs that predominantly give rise to neurons. These were early days, in 2002, and we wanted to do a transcriptional analysis to determine what was unique about this subset of cells that preferentially generated neurons. Affymetrix wasn't available then, there were no GeneChips to buy, so we used the entire genome for our screening, not just the expressed part. The Affymetrix chips mask a lot of the repeat sequences, but we didn't know enough to do that. When we compared the transcriptome of the NPCs with that of the more mature cell types, we found, to our surprise, that the top nine genes specifically expressed in NPCs were all sequences within LINE-1 retrotransposons. This finding was initially a little disturbing.

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ER: That they were the top hits does seem unexpected.

FG: Yes, this was not something that people were thinking about, certainly not in neurobiology. At that time, there was a certain amount of disappointment in my lab, and I was wondering whether we should just forget this finding and go down the list to hits that made a little more sense. However, there was growing interest from other members of the lab, and I was curious now, because this was the second time that I had seen these IAPs or mobile elements showing up in NPCs.

We began reading more about them, and the first thing we did was to try and detect these transcripts through standard procedures in the NPCs. We could detect them at high levels and we saw that in other cells their levels were lower. However, we quickly realized that transcription was not really the issue here. These were mobile elements, and what would be most interesting is if they actually inserted themselves into the DNA of the NPCs. At this time, and as I usually do when I'm thinking about looking at things that are new in my field, I asked the experts for advice. We contacted Haig Kazazian and John Moran, who had developed assay systems and were the leaders in the transposable element field, to discuss how to best approach this issue. They had developed a reporter construct that was the gold standard for measuring insertions into the genome of individual cells: any cell in which a retrotransposable element had mobilized would turn fluorescent green. We used this assay and, to our excitement, we saw that our NPCs were green. Not all of them, obviously.

ER: But there was some mobilization.

FG: Yes! There was mobilization, and it wasn't in fibroblasts. We looked at a variety of other cell types as controls and did a fair amount of *in vitro* work trying to gain some confidence in these results, but quickly realized that the significance of our finding would be higher if we could determine whether the transposable elements also moved *in vivo*. We made transgenic

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DOI 10.15252/embr.201439088 | Published online 1 July 2014

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animals with the reporter construct and saw green neurons in the adult animals: beautiful! It was a very exciting moment in the lab to see that. We didn't see this green fluorescence in other cell types or other tissues, except in germline tissue, which we didn't pursue very much at the time, because retrotransposition was known to occur in the germline.

We then looked developmentally and saw green cells in the embryos at the time that neurogenesis was taking place, so retrotransposition was not just an adult event but occurred during embryogenesis. Those results were reported in our first publication on the topic in *Nature*, which had a lot of information in it: the initial observation, the screening, the mobilization *in vitro* and *in vivo*. We also identified some integration hot spots, although the mobile elements were clearly not jumping into the same site each time. There was significant diversity of insertion sites, and it looked like there was a preference for neuronal genes, but we were quite cautious about that because neural genes are abundant in the genome, Furthermore, since we were looking at mobilization in NPCs, the chromatin was likely open at these sites, so there could be tissue culture artifacts. We presented the data, but didn't give much interpretation of the results. This article was somewhat controversial at the time.

ER: Then you turned to human cells?

FG: Yes, we wanted to test whether retrotransposition occurred in humans, because all of our previous work had been done in rodents. We had some human NPCs but, more importantly, human embryonic stem (ES) cells were now becoming available, and we were developing procedures for generating NPCs and neurons from human ES cells for other reasons. Using a humanized version of the reporter, we could detect retrotransposition in human NPCs *in vitro*.

Then the push came to determine if endogenous levels of retrotransposition could be measured. Whole-genome sequencing and RNA-Seq technology were not available, so we devised—in collaboration with many of our colleagues—primers that identified about 100 full-length LINEs that are thought to be the most active in the human genome, and we used as control other repeat or highly represented sequences in the genome that are not mobile. The theory was that if mobilization was occurring in humans, you would see a greater amount of LINE DNA in human brain. We could clearly detect a greater amount of LINE DNA in human brain than in liver, heart, and some other tissues that we analyzed.

ER: How is LINE activity regulated?

FG: We found both YY1 and MECP2 boundaries around SOX2 binding sites at the promoter of LINE elements. SOX2 is a transcription factor important for NPCs that suppresses LINE activity, and there was an island of MECP2 methylation right next to SOX2. We showed that SOX2 and MECP2 work cooperatively to help to silence LINE activity. When the cells differentiate and SOX2 is removed, there is a bit of

demethylation and then the highest LINE activity occurs.

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Importantly, we identified MECP2 as a really important suppressor, and when we made NPCs from *MECP2* knockout mice, they had a significantly greater amount of mobile element activity and insertions. There is a disease known as Rett syndrome in which mutations cause loss of MECP2 function, and we obtained post-mortem tissue from six patients. Compared to normal age-matched controls, there is a greater amount of DNA insertion into the brain tissue of Rett syndrome patients.

ER: Which means that mobilization is occurring, at least in humans who have a defect in the repressor?

FG: Right, and it is greater in Rett patients than in the brains of normal healthy individuals, but we could also see mobilization in the latter. So that gave us a little more confidence that what occurs in animal models is relevant also in humans. And then Geoff Faulkner, using a sophisticated genomic method, showed a lot of LINE activity in the hippocampus, clearly confirming that there are insertional events occurring in the brains of humans, which he reported in *Nature*. And last year, Chris Walsh reported in *Cell* that, using single cells, they could calculate that between one in two and one in fifty neurons in the brain had a unique somatic LINE insertion, and he considered this low. However, from our perspective, if there are 100 billion neurons in the brain, one in two means that 50 billion neurons have insertions in them in some place.

ER: Where is the field going now?

FG: People have started looking at stressed animals, such as after alcohol consumption, and at disease states, such as the recent report on schizophrenia in *Neuron*. More often now, transposable element mobilization is found to be increased in a variety of conditions.

From a quantitative perspective, we need to understand whether there are preferential regions of activity in the absence of disease or stress, whether there are integration hot spots. In this respect, we can gain a lot of insights from plant biology. The mobile element field has always been active in plant biology. A lot of my friends here at the Salk are plant biologists who are not surprised at all by our findings. They say they spend all their time mapping these things out, so they have been very helpful and encouraging in helping us map our insertions. The other folks that are really good in this area are the Drosophila investigators, who have been looking at LINEs and transposons for a long time. Here I should mention Scott Waddell and Josh Dubnau. Scott is at Oxford now and had a beautiful paper in *Science* showing that insertional mutagenesis of Gypsy in the mushroom body correlates with behavioral diversity in a learning task. It was really a great paper. And Josh had a paper in *Nature Neuroscience* showing that there is increased transposable element mobilization as flies age. He has also found that TDP-43—a protein altered in amyotrophic lateral sclerosis (ALS) and other neurodegenerative disorders—can suppress LINE activity. I know there is a lot of interest and ongoing research into the effects of retrotransposition in ALS and other diseases.

ER: Do you think that stress or homeostatic alterations activate these elements?

FG: That's the working hypothesis. I think it was actually Barbara McClintock who suggested that stress—in terms of climate change and things like that—would activate the variegation in corn transposons. So I don't attribute this idea to any of us recent investigators. You just have to go back to her literature to see that she already suggested that stress was involved. But what is not known, and is under intense investigation, is what we mean by stress, and the mechanism by which stress occurs. I'm very excited about this area; we have a couple of things we're working on right now, more mechanistically, about how that might happen.

There's another thing that should be recognized: the ribonucleoprotein complex (RNP) that makes up the LINE RNA and the two proteins that bind it, ORF1 and ORF2, cannot be imported back into the nucleus by themselves. As far as we know, in a quiescent cell—like a neuron—active transport is required to get across the nuclear pore. And the LINE RNP is a big complex, so as far as we know it could only access the chromatin during cell division. Most of these retrotransposition events are thus likely to have occurred early in development and are being detected in the adult when some kind of stress event activates the promoter, but it's unlikely that it activates a new insertion.

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ER: Is this mobilization therefore a consequence of, or somehow driving these conditions?

FG: I think we just don't know enough right now, and the safest statement is to say it is a consequence. For example, from our studies on Rett syndrome and ataxia telangiectasia mutated (ATM), the most parsimonious explanation is that the disease and the mutations, respectively, activate the LINE elements. So transposable element mobilization is a consequence of these diseases, it is not causing the disease. And, I don't even know whether it is beneficial or detrimental.

ER: Indeed, because speciation and adaptation all come from this, right? From increased mutagenesis, increased variability can be negative, but also can be positive.

FG: You know, that's exactly how I see it. This is a general mechanism for generating diversity; it's genes in conflict, the traditional story of human evolution or of species evolution. Something happens to the genome that confers a protective or beneficial trait and is thus selected for. I was at an epigenetic meeting recently, and some people thought that the only reason epigenetic mechanisms evolved was to block LINE and SINE activity. They were saying that this is what mobile elements do, what they are all about. I wouldn't go as far as that, but it may well be that they did in part evolve to protect the genome, or regulate the mobility of SINEs, LINEs and other elements within the genome. This doesn't mean they have not been exapted; epigenetics could have been exapted for other purposes, such as modulation of behavior and stress response, which happens all the time.

ER: What do you think are the evolutionary implications of transposable element mobilization?

FG: I am very interested in human evolution. We have a large group of researchers here in San Diego that has now become an international group that meets three times a year and looks at various aspects of how it is that we became human. These are archaeologists, genomicists, language specialists; a wide variety of disciplines are included. It's really quite fascinating. It's called the Center for Academic Research and Training in Anthropogeny (CARTA); our webpage gives you a full flavor of the kinds of things that we do (carta.anthropogeny.org).

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One thing that was missing in the discussion for me was having some cell-based assay to do molecular biology to answer some of the evolutionary questions that were emerging from our meetings. At the time, we were starting to work on human ES cells, and it occurred to me and a few colleagues in my lab, including Carol Marchetto and Alysson Muotri, to make ES cells from all the major primates, because then we could make NPCs, and neurons and compare neurons from different species. Getting sperm and egg from chimpanzees and bonobos was very, very difficult, and the procedure of somatic cell nuclear transfer is not straightforward. Not only that, but we had approval problems to obtain sperm or eggs from the zoos, because bonobos, chimps, and many other apes are protected animals. Serendipitously, the development of induced pluripotent stem (iPS) cell technology opened the door for us. It still took several years to obtain fibroblasts from all the major apes from zoos, but we now have them from human, chimp, bonobo, orangutan, gorilla, rhesus, and marmoset, and we are generating iPS cells. Initially, we just looked at iPS cells generated from human, chimp, and bonobo, because there was a growing suggestion that there are differences in the diversity of the genomes of humans compared to chimps and bonobos. For example, a study analyzing the total amount of mitochondrial DNA diversity in chimps in East Africa has shown that there is more diversity between two troops on either side of a mountain range than among all the human species, including Neanderthal.

ER: Wow, so speciation is huge in chimps.

FG: Huge and still growing. So we wanted to uncover the mechanism that might be driving this diversity. We sequenced the transcriptomes of the iPS cells of human, chimp, and bonobo (multiple animals and multiple clones by RNA Seq) and figured that any event that occurs in these cells would be passed along in the germ line, and thus generate diversity. A lot of interesting things came out of this analysis. First of all, we could easily detect differences of humans from chimps and bonobos, and we showed that the iPS cells from chimps and bonobos were very similar to each other. There were about 500 genes that had a significantly different expression level between humans, chimps, and bonobos, but we focused on the top 50 that were highly different. Although you can do all kinds of bioinformatics, we looked through the list and picked out our favorite genes to work on. Two of the ones that showed up were *APOBEC3B* and *PIWIL2*. Both of them seemed to be expressed 10 to 20 times more in humans than in chimps and bonobos. We liked these two genes—and this was just a selection, there are more—because there is a strong literature showing that they suppress LINE activity. We used our reporter assays to show that humans have much less insertion—there is a little bit, but chimps and bonobos have massive amounts of insertions of new elements into their genome. We did a lot of mechanistic work and, in the end, we examined all the LINE sequences in the genomes of chimp versus human that are available in the genome browser. Remember that LINEs have been evolving stepwise over the last 30 million years, and we could see no difference in the total amount of DNA sequence for any of the older LINE elements; they are all equivalent in human and chimp. But for the most recent LINEs, there are thousands more DNA sequences in the chimp relative to the human. Chimps have a greater amount of insertions, whereas mobilization in our genome seems to be suppressed.

"Chimps have a greater amount of [recent LINE DNA] insertions, whereas mobilization in our genome seems to be suppressed."

These findings are now being confirmed by other groups. Evan Eichler has a study showing many more single nucleotide variations in chimps than in humans. We, as humans, have a more homogeneous, less diversified genome compared to each other. You and I—although unrelated—may be much more like each other than a brother and sister chimp would be. The consequence of this lack of diversity, and this is the hypothesis or speculation, is that it is actually dangerous to us to some extent. Humans have less diversity; we are not diversifying as much as the chimps and bonobos.

ER: Our capacity for adaptation is less, then?

FG: Our genetic capacity for adaptation is less, so we are phenotypically more similar to each other. This similarity allows us to more easily communicate and transmit information with each other and across generations, and we become more dependent on cultural evolution to respond to changes in the environment than we are on genetic evolution.

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For example, Picasso and Braque discovered Cubism, and I would attribute their uniqueness, their genius, to doing that. However, once they had developed it, because of cultural evolution, the rest of us can incorporate Cubism in our work, as it has been in modern art. It is reflected in many things—architecture, painting,

everywhere—and we don't need the genome of Picasso or Braque to propagate that; we are using cultural evolution to propagate our knowledge.

One can imagine that the reason that the genetic diversity in humans narrowed so much was due, for example, to some viral infection as they migrated out of Africa. However, we are in danger, as a species—like all other species—of these environmental impacts, such as viral infections, that could wipe our species out. If we relied solely on genomic variation for species survival, we would have high and low sweeps through our population; we probably wouldn't be evolving. But we have adapted another mechanism, a genome-independent mechanism. What do we do instead? We build hospitals, we train scientists, we come up with mechanisms to fight disease from a cultural perspective, rather than evolving into another human species slightly different from ourselves that is protected from that specific virus or other potentially lethal pathogen. I know this is a speculation, but this is how we are thinking about it.

ER: But it's really interesting because, for example, the current rise in metabolic disease has been partly attributed to an insufficient evolutionary time to adapt to our changes in lifestyle. Perhaps if we were more genetically variable, we would adapt faster, but because we are not, these changes—that do need to be genetic to modify metabolic activity—have not yet occurred.

FG: Exactly. We're stuck in a hunter-gatherer kind of metabolic activity.

ER: I would like to touch on one last point. You have a unique perspective about dogmas in biology, because you have broken a couple by showing the existence of adult neurogenesis and of somatic retrotransposition in animals. Was there a walk through the desert for you? How do you feel about dogmas in biology?

FG: This is a really good question, and I don't have a good answer. I would first point out that I didn't set out to overturn dogma. I got to that point by studying something that was very interesting to me and that I felt I could make a unique contribution to. For example, early on, people were actively against the idea that there was any neurogenesis in the adult nervous system. They said that there was likely some technical mistake. This argument forced us to develop new techniques. Going into new fields requires a lot of building of new technology, working with the experts to expand on what is known, to develop more sensitive assays. We developed a lot of techniques and handed them to our most ardent detractors, asking them whether they could reproduce what we saw.

I sometimes tell my students and post-docs that the first time you find something that's different than what's expected, people say that you made a mistake and that you need to go back to your data and get better tools, and they ignore your finding. Then you show them more results, and they get a little angry sometimes, or tell you that you shouldn't be doing this. And when they finally accept that it's possible, sometimes they contend that it's trivial. They admit that, it does happen, but to a very limited extent, and that it is only detectable because the technology has improved, and it can't possibly be of any functional significance or meaning. Then there is this transient period of time in which people finally accept that it happens, and it looks like it might have some small but clear function in biology. And then, a moment later, they say "oh yeah, I knew that, everybody knows that!" That's my take on the process of discovering something new. It's great fun.

For me, there have been these transition points, such as when we saw these LINE sequences in the initial transcriptional analyses. I then asked myself whether we were really going to go after this. We started really just nibbling; we would do a few experiments and just kept trying to be consistent. We tried to prove ourselves wrong but couldn't, so we kept following down that path. I have been very fortunate to have very smart, creative, adventurous post-docs and graduate students working in my lab, who then in their own independent labs have done superb work that extends beyond what I've done. We have a lot of fun going after these lively topics in science.

ER: Taking your experience into consideration, how would you advise young people to approach discovery?

FG: I tell young starting scientists to find something that they are passionate about and in which they can uniquely make a difference. I advise them to not necessarily follow trends, and if they are entering a new field, find the experts and get as much information as they can about the right way to do things. Use the best techniques possible, and if the techniques are not good enough, then devise new methods and techniques.

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The most important thing is to try to prove yourself wrong. Often in science people avoid those tests that are going to prove your favorite theory completely wrong. I tell my students that, when you are doing science, you are not trying to prove your hypothesis to be correct, you are trying to prove it wrong. That's what strengthens your hypothesis, to take away those pieces that lead to questioning whether it's correct or not. And, above all, make sure that you really enjoy it.

ER: Thank you very much Rusty, it was a real pleasure.

This interview was conducted by Nonia Pariente.