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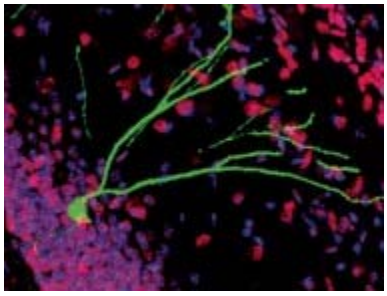
Article Title:
'Jumping Genes' Linked to Schizophrenia
January 02, 2014
Emily Underwood



Carol Marchetto (Salk Institute) and Alysson Muotri (UCSD)

Risk and reward. "Jumping genes" (in green neuron) may help ensure that every brain is unique, but could also contribute to neurological disorders such as schizophrenia.

Roaming bits of DNA that can relocate and proliferate throughout the genome, called "jumping genes," may contribute to schizophrenia, a new study suggests. These rogue genetic elements pepper the brain tissue of deceased people with the disorder and multiply in response to stressful events, such as infection during pregnancy, which increase the risk of the disease. The study could help explain how genes and environment work together to produce the complex disorder and may even point to ways of lowering the risk of the disease, researchers say.

Schizophrenia causes hallucinations, delusions, and a host of other cognitive problems, and afflicts roughly 1% of all people. It runs in families—a person whose twin sibling has the disorder, for example, has a roughly 50-50 chance of developing it. Scientists have struggled to define which genes are most important to developing the disease, however; each individual gene associated with the disorder confers only modest risk. Environmental factors such as viral infections before birth have also been shown to increase risk of developing schizophrenia, but how and whether these exposures work together with genes to skew brain development and produce the disease is still unclear, says Tadafumi Kato, a neuroscientist at the RIKEN Brain Science Institute in Wako City, Japan and co-author of the new study.

Over the past several years, a new mechanism for genetic mutation has attracted considerable interest from researchers studying neurological disorders, Kato says. Informally called jumping genes [1], these bits of DNA can replicate and insert themselves into other regions of the genome, where they either lie silent, doing nothing; start churning out their own genetic products; or alter the activity of their neighboring genes. If that sounds potentially dangerous, it is: Such genes are often the culprits behind tumor-causing mutations and have been implicated in several neurological diseases. However, jumping genes also make up nearly half the current human genome, suggesting that humans owe much of our identity to their audacious leaps.

Recent research by neuroscientist Fred Gage and colleagues at the University of California (UC), San Diego, has shown that one of the most common types of jumping gene in people,

called L1, is particularly abundant in human stem cells in the brain that ultimately differentiate into neurons and plays an important role in regulating neuronal development and proliferation [2]. Although Gage and colleagues have found that increased L1 is associated with mental disorders such as Rett syndrome, a form of autism, and a neurological motor disease called Louis-Bar syndrome, "no one had looked very carefully" to see if the gene might also contribute to schizophrenia, he says.

To investigate that question, principal investigator Kazuya Iwamoto, a neuroscientist; Kato; and their team at RIKEN extracted brain tissue of deceased people who had been diagnosed with schizophrenia as well as several other mental disorders, extracted DNA from their neurons, and compared it with that of healthy people. Compared with controls, there was a 1.1-fold increase in L1 in the tissue of people with schizophrenia [3], as well as slightly less elevated levels in people with other mental disorders such as major depression, the team reports today in *Neuron*.

Next, the scientists tested whether environmental factors associated with schizophrenia could trigger a comparable increase in L1. They injected pregnant mice with a chemical that simulates viral infection and found that their offspring did, indeed, show higher levels of the gene in their brain tissue. An additional study in infant macaques, which mimicked exposure to a hormone also associated with increased schizophrenia risk, produced similar results. Finally, the group examined human neural stem cells extracted from people with schizophrenia and found that these, too, showed higher levels of L1.

The fact that it is possible to increase the number of copies of L1 in the mouse and macaque brains using established environmental triggers for schizophrenia shows that such genetic mutations in the brain may be preventable if such exposures can be avoided, Kato says. He says he hopes that the "new view" that environmental factors can trigger or deter genetic changes involved in the disease will help remove some of the disorder's stigma.

Combined with previous studies on other disorders, the new study suggests that L1 genes are indeed more active in the brain of patients with neuropsychiatric diseases, Gage says. He cautions, however, that no one yet knows whether they are actually causing the disease. "Now that we have multiple confirmations of this occurring in humans with different diseases, the next step is to determine if possible what role, if any, they play."

One tantalizing possibility is that as these restless bits of DNA drift throughout the genomes of human brain cells, they help create the vibrant cognitive diversity that helps humans as a species respond to changing environmental conditions, and produces extraordinary "outliers," including innovators and geniuses such as Picasso, says UC San Diego neuroscientist Alysson Muotri. The price of such rich diversity may be that mutations contributing to mental disorders such as schizophrenia sometimes emerge. Figuring out what these jumping genes truly do in the human brain is the "next frontier" for understanding complex mental disorders, he says. "This is only the tip of the iceberg."

Links:

[1] http://news.sciencemag.org/paleontology/2010/07/jumping-genes-shed-light-marsupial-migration
[2] http://www.sciencemag.org/content/332/6027/300
[3] http://dx.doi.org/10.1016/j.neuron.2013.10.053

The Loom

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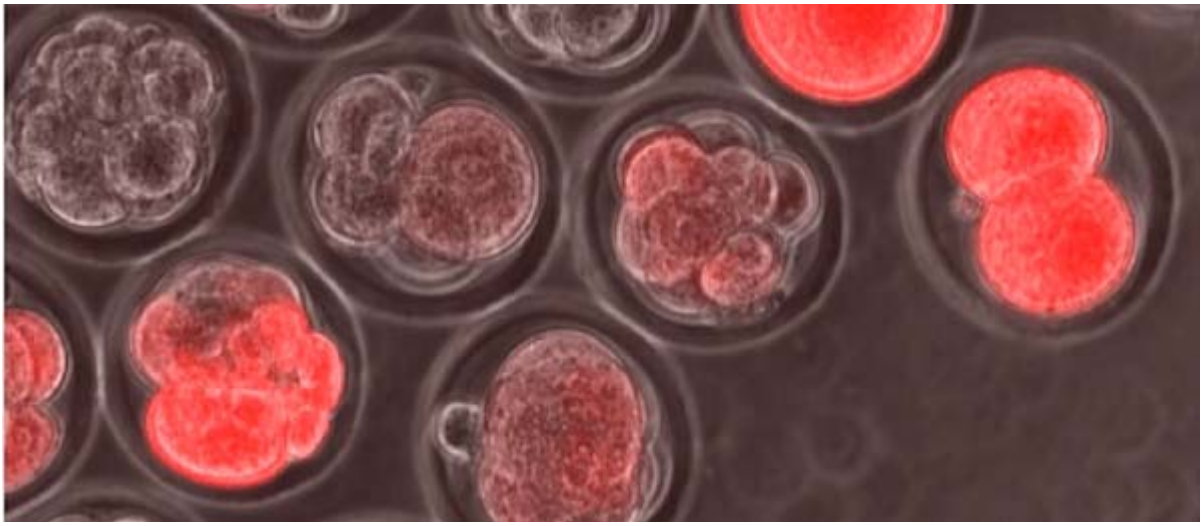
We Are Viral From the Beginning

By Carl Zimmer | June 14, 2012 1:42 pm



We all started out as a fertilized egg: a solitary cell about as wide as a shaft of hair. That primordial sphere produced the ten trillion cells that make up each of our bodies. We are not merely sacs of identical cells, of course. A couple hundred types of cells arise as we develop. We're encased in skin, inside of which bone cells form a skeleton; inside the skull are neurons woven into a brain.

What made this alchemy possible? The answer, in part, is viruses.

Viruses are constantly swarming into our bodies. Sometimes they make us sick; sometimes our immune systems vanquish them; and sometimes they become a part of ourselves. A type of virus called a retrovirus makes copies of itself by inserting its genes into the DNA of a cell. The cell then uses those instructions to make the parts for new viruses. HIV makes a living this way, as do a number of viruses that can trigger cancer.

On rare occasion, a retrovirus may infect an egg. Now something odd may happen. If the egg becomes fertilized and gives rise to a whole adult individual, all the cells in its body will carry that virus. And if that individual has offspring, the virus gets carried down to the next generation.

At first, these so-called endogenous retroviruses lead a double life. They can still break free of their host and infect new ones. Koalas are suffering from one such epidemic. But over thousands of years, the viruses become imprisoned. Their DNA mutates, robbing them of the ability to infect new hosts. Instead, they can only make copies of their genes that are then inserted back into their host cell. Copy after copy build up the genome. To limit the disruption these viruses can cause, mammals produce proteins that can keep most of them locked down. Eventually, most endogenous retroviruses mutate so much they are reduced to genetic baggage, unable to do anything at all. Yet they still bear all the hallmarks of viruses, and are thus recognizable to scientists who sequence genomes. It turns out that the human genome contains about 100,000 fragments of endogenous retroviruses, making up about eight percent of all our DNA.

Evolution is an endlessly creative process, and it can turn what seems utterly useless into something valuable. All the viral debris scattered in our genomes turns out to be just so much raw material for new adaptations. From time to time, our ancestors harnessed virus DNA and used it for our own purposes. In a new paper in the journal Nature, a scientist named Samuel Pfaff and a group of fellow scientists report that one of those purposes to help transform eggs into adults.

In their study, Pfaff and his colleagues at the Salk Institute for Biological Sciences examined fertilized mouse eggs. As an egg starts to divide, it produces new cells that are capable of becoming any part of the embryo–or even the membrane that surrounds the embryo or the placenta that pipes in nutrients from the animal's mother. In fact, at this early stage, you can pluck a single cell from the clump and use it to grow an entire organism. These earliest cells are called totipoent.

After a few days, the clump becomes a hollowed out ball. The cells that make the ball up are still quite versatile. Depending on the signals a cell gets at this point, it can become any cell type in the body. But once the embryo reaches this stage, its cells have lost the ability to give rise to an entirely new organism on their own, because they can't produce all the extra tissue required to keep an embryo alive. Now the cells are called pluripotent. The descendants of pluripotent cells gradually lose their versatility and get locked into being certain types of cells. Some become hematopoetic cells, which can turn into lots of different kinds of blood cells but can no longer become, say, skin cells.

Pfaff and his colleagues examined mouse embryos just after they had divided into two cells, in the prime of their totipotency. They catalogued the genes that were active at that time–genes which give the cells their vastly plastic potential. They found over 100 genes that were active at the two-cell stage, and which then shut down later on, by the time the embryo had become a hollow ball.

One way cells can switch genes on and off is producing proteins that latch onto nearby stretches of DNA called promoters. The match between the protein and the promoter has to be precise; otherwise, genes will be flipping on at all the wrong times, and failing to make proteins when they're needed. Pfaff and his colleagues found that all the two-cell genes had identical promoters–which would explain how they all managed so become active at the same time.

What was really remarkable about their discover was the origin of those promoters. They came from viruses.

During the earliest stage of the embryo's development, these virus-controlled genes are active. Then the cells clamp down on them, just as they would clamp down on viruses. Once those genes are silenced, the totipotent cells become pluripotent.

Pfaff and his colleagues also discovered something suprising when they looked at the pluripotent ball of cells. From time to time, the pluripotent cells let the virus-controlled genes switch on again, and then shut them back down. All of the cells, it turns out, cycle in and out of what the scientists call a "magic state," in which they become temporarily totipotent again. (The pink cells in this photo are temporarily in that magic state.)

Cells in the magic state can give rise to any part of the embryo, as well as the placenta and other tissue outside the embryo. Once the virus-controlled genes get shut down again, they lose that power. This discovery demonstrated that these virus-controlled genes really are crucial for making cells totipotent.

Pfaff and his colleagues propose that the domestication of these virus promoters was a key step in the evolution of mammals with placentas. The idea that viruses made us who were are today may sound

bizarre, except that Pfaff is hardly the first person to find evidence for it. Last year, for example, I wrote about how placental mammals stole a virus protein to build the placenta.

A discovery this strange inevitably raises questions that its discoverers cannot answer. What are the virus-controlled genes doing in those first two cells? Nobody knows. How did the domestication of this viral DNA help give rise to placental mammals 100 million years ago? Who knows? Why are viruses so intimately involved in so many parts of pregnancy? Awesome question. A very, very good question. Um, do we have any other questions?

We don't have to wait to get all the answers to those questions before scientists can start to investigate one very practical application of these viruses. In recent years, scientists have been reprogramming cells taken either from adults or embryos, trying to goose them back into an early state. By inducing cells to become stem cells, the researchers hope to develop new treatments for Parkinson's disease and other disorders where defective cells need to be replaced. Pfaff suggests that we should switch on these virus-controlled genes to help push cells back to a magic state.

If Pfaff's hunch turns out to be right, it would be a delicious triumph for us over viruses. What started out as an epidemic 100 million years ago could become our newest tool in regenerative medicine.

(For more on these inner passengers, see my book *A Planet of Viruses*.)

[Image: Courtesy Salk Institute.]

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John S. Wilkins

Typo: totipoent.

I think that from the organismal lineage's perspective, a virus is just another kind of mutation. Despite the somatic hypermutation hypothesis, I have a hard time thinking that the frequency of beneficial ERV genes will be all that great relative to other kinds of mutation.

June 14, 2012 at 5:26 pm

John. The questions of the frequency and the importance of these viruses

June 14, 2012 at 5:54 pm

FROM THE JUNE 2010 ISSUE

The Insanity Virus

Schizophrenia has long been blamed on bad genes or even bad parents. Wrong, says a growing group of psychiatrists. The real culprit, they claim, is a virus that lives entwined in every person's DNA.

By Douglas Fox | Monday, November 08, 2010

RELATED TAGS: MENTAL HEALTH, GENES & HEALTH

Viruses like influenza or measles kill cells when they infect them. But when retroviruses like HIV infect a cell, they often let the cell live and splice their genes into its DNA. When the cell divides, both of its progeny carry the retrovirus's genetic code in their DNA.

In the past few years, geneticists have pieced together an account of how Perron's retrovirus entered our DNA. Sixty million years ago, a lemurlike animal—an early ancestor of humans and monkeys—contracted an infection. It may not have made the lemur ill, but the retrovirus spread into the animal's testes (or perhaps its ovaries), and once there, it struck the jackpot: It slipped inside one of the rare germ line cells that produce sperm and eggs. When the lemur reproduced, that retrovirus rode into the next generation aboard the lucky sperm and then moved on from generation to generation, nestled in the DNA. "It's a rare, random event," says Robert Belshaw, an evolutionary biologist at the University of Oxford in England. "Over the last 100 million years, there have been only maybe 50 times when a retrovirus has gotten into our genome and proliferated."

But such genetic intrusions stick around a very long time, so humans are chockablock full of these embedded, or endogenous, retroviruses. Our DNA carries dozens of copies of Perron's virus, now called human endogenous retrovirus W, or HERV-W, at specific addresses on chromosomes 6 and 7.

If our DNA were an airplane carry-on bag (and essentially it is), it would be bursting at the seams. We lug around 100,000 retrovirus sequences inside us; all told, genetic parasites related to viruses account for more than 40 percent of all human DNA. Our body works hard to silence its viral stowaways by tying up those stretches of DNA in tight stacks of proteins, but sometimes they slip out. Now and then endogenous retroviruses switch on and start manufacturing proteins. They assemble themselves like Lego blocks into bulbous retroviral particles, which ooze from the cells producing them.

Endogenous retroviruses were long considered genetic fossils, incapable of doing anything interesting. But since Perron's revelation, at least a dozen studies have found that HERV-W is active in people with MS.

By the time Perron made his discovery, Torrey and Yolken had spent about 15 years looking for a pathogen that causes schizophrenia. They found lots of antibodies but never the bug itself. Then Håkan Karlsson, who was a postdoctoral fellow in Yolken's lab, became interested in studies showing that retroviruses sometimes triggered psychosis in AIDS patients. The team wondered if other retroviruses might cause these symptoms in separate diseases such as schizophrenia. So they used an experiment, similar to Perron's, that would detect any retrovirus (by finding sequences encoding reverse transcriptase enzyme)—even if it was one that had never been catalogued before. In 2001 they nabbed a possible

culprit. It turned out to be HERV-W.

Several other studies have since found similar active elements of HERV-W in the blood or brain fluids of people with schizophrenia. One, published by Perron in 2008, found HERV-W in the blood of 49 percent of people with schizophrenia, compared with just 4 percent of healthy people. "The more HERV-W they had," Perron says, "the more inflammation they had." He now sees HERV-W as key to understanding many cases of both MS and schizophrenia. "I've been doubting for so many years," he says. "I'm convinced now."

Torrey, Yolken, and Sarven Sabunciyan, an epigeneticist at Johns Hopkins, are working to understand how endogenous retroviruses can wreak their havoc. Much of their research revolves around the contents of a nondescript brick building near Washington, D.C. This building, owned by the Stanley Medical Research Institute, maintains the world's largest library of schizophrenic and bipolar brains. Inside are hundreds of cadaver brains (donated to science by the deceased), numbered 1 through 653. Each brain is split into right and left hemispheres, one half frozen at about –103 degrees Fahrenheit, the other chilled in formaldehyde. Jacuzzi-size freezers fill the rooms. The roar of their fans cuts through the air as Torrey's team examines the brains to pinpoint where and when HERV-W awakens into schizophrenia.

New high-speed DNA sequencing is making the job possible. In a cramped room at Johns Hopkins Medical Center, a machine the size of a refrigerator hums 24/7 to read gene sequences from samples. Every few minutes the machine's electric eye scans a digital image of a stamp-size glass plate. Fixed to that plate are 300 million magnetic beads, and attached to each bead is a single molecule of DNA, which the machine is sequencing. In a week the machine churns out the equivalent of six human genomes —enough raw data to fill 40 computer hard drives.

The hard part starts when those sequences arrive at Sabunciyan's desk. "We got these data right around New Year's 2009," Sabunciyan said one day last August as he scrolled through a file containing 2 billion letters of genetic code, equivalent to 2,000 John Grisham novels composed just of the letters G, A, T, and C (making the plot a great deal more confusing). "We're still looking at it."

Sabunciyan has found that an unexpectedly large amount of the RNA produced in the brain—about 5 percent—comes from seemingly "junk" DNA, which includes endogenous retroviruses. RNA is a messenger of DNA, a step in the path to making proteins, so its presence could mean that viral proteins are being manufactured in the body more frequently than had been thought.

Through this research, a rough account is emerging of how HERV-W could trigger diseases like schizophrenia, bipolar disorder, and MS. Although the body works hard to keep its ERVs under tight control, infections around the time of birth destabilize this tense standoff. Scribbled onto the marker board in Yolken's office is a list of infections that are now known to awaken HERV-W—including herpes, toxoplasma, cytomegalovirus, and a dozen others. The HERV-W viruses that pour into the newborn's blood and brain fluid during these infections contain proteins that may enrage the infant immune system. White blood cells vomit forth inflammatory molecules called cytokines, attracting more immune cells like riot police to a prison break. The scene turns toxic.

In one experiment, Perron isolated HERV-W virus from people with MS and injected it into mice. The mice became clumsy, then paralyzed, then died of brain hemorrhages. But if Perron depleted the mice of immune cells known as T cells, the animals survived their encounter with HERV-W. It was an extreme experiment, but to Perron it made an important point. Whether people develop MS or schizophrenia may depend on how their immune system responds to HERV-W, he says. In MS the immune system directly attacks and kills brain cells, causing paralysis. In schizophrenia it may be that inflammation damages neurons indirectly by overstimulating them. “The neuron is discharging neurotransmitters, being excited by these inflammatory signals,” Perron says. “This is when you develop hallucinations, delusions, paranoia, and hyper-suicidal tendencies.”

The first, pivotal infection by toxoplasmosis or influenza (and subsequent flaring up of HERV-W) might happen shortly before or after birth. That would explain the birth-month effect: Flu infections happen more often in winter. The initial infection could then set off a lifelong pattern in which later infections reawaken HERV-W, causing more inflammation and eventually symptoms. This process explains why schizophrenics gradually lose brain tissue. It explains why the disease waxes and wanes like a chronic infection. And it could explain why some schizophrenics suffer their first psychosis after a mysterious, monolike illness.

The infection theory could also explain what little we know of the genetics of schizophrenia. One might expect that the disease would be associated with genes controlling our synapses or neurotransmitters. Three major studies published last year in the journal *Nature* tell a different story. They instead implicate immune genes called human leukocyte antigens (HLAs), which are central to our body’s ability to detect invading pathogens. “That makes a lot of sense,” Yolken says. “The response to an infectious agent may be why person A gets schizophrenia and person B doesn’t.”

Gene studies have failed to provide simple explanations for ailments like schizophrenia and MS. Torrey’s theory may explain why. Genes may come into play only in conjunction with certain environmental kicks. Our genome’s thousands of parasites might provide part of that kick.

“The ‘genes’ that can respond to environmental triggers or toxic pathogens are the dark side of the genome,” Perron says. Retroviruses, including HIV, are known to be awakened by inflammation—possibly the result of infection, cigarette smoke, or pollutants in drinking water. (This stress response may be written into these parasites’ basic evolutionary strategy, since stressed hosts may be more likely to spread or contract infections.) The era of writing off endogenous retroviruses and other seemingly inert parts of the genome as genetic fossils is drawing to an end, Perron says. “It’s not completely junk DNA, it’s not dead DNA,” he asserts. “It’s an incredible source of interaction with the environment.” Those interactions may trigger disease in ways that we are only just beginning to imagine.

Torrey’s sister has had a tough go of it. Schizophrenia treatments were limited when she fell ill. Early on she received electroshock therapy and insulin shock therapy, in which doctors induced a coma by lowering her blood sugar level. Rhoda Torrey has spent 40 years in state hospitals. The disease has left only one part of her untouched: Her memory of her brief life before becoming ill—of school dances and sleepovers half a century ago—remains as clear as ever.

Steven Elmore was more fortunate. Drug therapy was widely available when he fell ill, and although he still hears voices from time to time, he has done well. Now 50 years old, he is married, cares for an adopted son and stepson, and works full time. He has avoided common drug side effects like diabetes, although his medications initially caused him to gain 40 pounds.

Torrey and Yolken hope to add a new, more hopeful chapter to this story. Yolken's wife, Faith Dickerson, is a clinical psychologist at Sheppard Pratt Health System in Baltimore. She is running a clinical trial to examine whether adding an anti-infective agent called artemisinin to the drugs that patients are already taking can lessen the symptoms of schizophrenia. The drug would hit HERV-W indirectly by tamping down the infections that awaken it. "If we can treat the toxoplasmosis," Torrey says, "presumably we can get a better outcome than by treating [neurotransmitter] abnormalities that have occurred 14 steps down the line, which is what we're doing now."

Looking ahead, better prenatal care or vaccinations could prevent the first, early infections that put some people on a path to schizophrenia. For high-risk babies who do get sick, early treatment might prevent psychosis from developing two decades later. Recent work by Urs Meyer, the neuroimmunologist, and his colleague Joram Feldon at the Swiss Federal Institute of Technology drives this point home. When they injected pregnant mice with RNA molecules mimicking viral infections, the pups grew up to resemble schizophrenic adults. The animals' memory and learning were impaired, they overreacted to startling noises, and their brain atrophied. But this March, Meyer and Feldon reported that treating the baby mice with antipsychotic drugs prevented them from developing some of these abnormalities as adults.

Perron has founded a biotech start-up —GeNeuro, in Geneva, Switzerland—to develop treatments targeting HERV-W. The company has created an antibody that neutralizes a primary viral protein, and it works in lab mice with MS. "We have terrific effects," Perron says. "In animals that have demyelinating brain lesions induced by these HERV envelope proteins, we see a dramatic stop to this process when we inject this antibody." He is scheduled to begin a Phase 1 clinical trial in people with MS near the end of this year. A clinical trial with schizophrenics might follow in 2011.

Even after all that, many medical experts still question how much human disease can be traced to viral invasions that took place millions of years ago. If the upcoming human trials work as well as the animal experiments, the questions may be silenced—and so may the voices of schizophrenia.

Minireview

Koala retrovirus: a genome invasion in real time

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The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2006/7/11/241

Abstract

Koalas are currently undergoing a wave of germline infections by the retrovirus KoRV. Study of this phenomenon not only provides an opportunity for understanding the processes regulating retrovirus endogenization but may also be essential to preventing the extinction of the species.

The genomes of all higher organisms are littered with the remnants of past retroviral infections, some dating back many tens of millions of years. The unique replication cycle of retroviruses, involving the integration of viral genetic information into host-cell DNA as a provirus, allows the formation of a permanent association between the virus and the infected cell. If the infected cell is a germ cell, then that genetic association can persist for many generations, with the provirus forming part of the genome of every cell in progeny.

Until now, we have never had the opportunity of observing or studying such genomic colonization as it takes place. Enter the koala - an Australian icon and a potentially endangered species. A recent paper in *Nature* by Tarlington and colleagues [1] provides evidence that koalas are in the midst of a germline invasion by the koala retrovirus (KoRV). They show that KoRV is present, at variable copy number, in the germline of all koalas found in Queensland, but that animals from some areas of southern Australia lack the provirus. Most notably, KoRV appears completely absent from koalas on Kangaroo Island off the coast of South Australia. This island was stocked with koalas in the early part of the twentieth century and has remained essentially isolated since then; it appears most likely that the small founding population was entirely free of KoRV. Tarlington *et al.* [1] suggest that an ongoing process of infection and endogenization is now occurring, spreading from a focus in northern Australia that quite possibly initiated within the last 100 to 200 years. Studies of the origin, properties and growth of KoRV may provide invaluable insights into the factors influencing retroviral endogenization.

Retroviruses as a fossil record

Inherited proviruses, or endogenous retroviruses (ERVs), are inherited in Mendelian fashion, and thus can provide a 'fossil record' for vertebrate infection by retroviruses [2]. Individual integration events can be distinguished by the cellular sequences flanking the provirus, outside the long terminal repeats (LTRs) that characterize each provirus; for example, a provirus at a common site in two related species implies an insertion event pre-dating the evolutionary split between the species. Studies of primate ERVs indicate an ongoing process of retrovirus acquisition for a period in excess of 30 million years [3]. Analysis of the human genome sequence reveals the presence of between 30 and 40 phylogenetic groups of viruses, ranging in prevalence from 1 copy to more than 1,000. Each group is thought to descend from one cross-species infection, followed by a series of amplification events, most probably including re-infection [4]. Indeed, it appears that proviruses make up a greater fraction of the human genome (6 to 8%) than do protein-coding sequences (1 to 2%) [5]. Only a minute fraction of the inherited proviruses can encode functional retroviruses, as all have suffered mutational decay to an extent related to their period of residence in the genome. Nevertheless, ERVs are associated with a wide range of biological phenomena, including neoplasia. The replication properties of retroviruses and the structures and distribution of proviruses in the germline allow us to infer the likely course of events during a wave of endogenization, but until now the process has not lent itself to experimental study [2]. The ongoing infection of koalas presents an opportunity to remedy that situation.

KoRV was originally described as an endogenous retrovirus based on its ubiquitous presence in all koala samples initially examined [6]. However, unlike most ERVs, KoRV appeared biologically active with ready demonstration of viral particles from cultured koala lymphocytes [6] and significant variation of KoRV copy number [7]. These observations prompted Tarlington *et al.* [1] to investigate the distribution and properties of KoRV in more detail. On the one hand, consistent with the proposition that KoRVs are endogenous, they could show the presence of viral sequences in sperm by fluorescent *in situ* hybridization and demonstrate Mendelian inheritance of specific proviruses in related individuals by Southern hybridization. On the other hand, variation in the KoRV envelope gene sequence was consistent with the propagation of exogenous KoRV. Furthermore, there was considerable variation in the proviral content of unrelated animals, implying that these elements had not been present in the germline for sufficient time to allow genetic fixation.

Studies of koala samples from different geographic locations suggest an on-going process of endogenization spreading from the north of Australia, where all animals contain endogenous KoRV, to the south, where some animals are still virus-free. Setting an accurate time for the start of this epidemic remains a problem; on the basis of the similarity of KoRV to an exogenous virus (one that is not integrated into the germline), called Gibbon Ape Leukemia Virus (GALV), Tarlington *et al.* [1] conclude that it occurred less than 100 years ago. However, this may be an underestimate given the difficulties of determining rates of retrovirus evolution [8]. PCR examination of preserved koala DNA, if any suitable specimens can be identified, might provide a means of addressing this question.

Where did KoRV come from?

Six genera of retroviruses are currently recognized; of these, at least two - deltaviruses and lentiviruses - appear never to have generated ERVs. Although this particular observation may have a fairly trivial explanation, namely the absence of specific receptors for these viruses on germ cells, it does prompt more global questions about the characteristics required for cross-species infection and whether virus evolution either before or after initial colonization is required for successful invasion of the germline. One approach to examining these questions would be to compare the biological properties of the virus that initiates invasion in a species with the one that emerges as a stable ERV, along with any intermediates that can be found. For most ERVs, the progenitor viruses are lost in evolutionary time and cannot be studied, but this approach may be feasible with the KoRVs, due to the relatively recent colonization.

A starting point for the search for the origin of KoRV is its sequence relationship to GALV [6]. Older, pre-genomic studies indicated that GALV in turn is derived from an endogenous retrovirus of the Asian mouse *Mus caroli* or a related species [9]. Using more modern techniques, the hunt is currently under way for one or more viruses from these mice that are closely related to KoRV, and for mammalian vectors that might have allowed the transmission of a virus from mice in Southeast Asia to koalas in Australia. In another paper published recently on the characterization of the koala retrovirus, Oliveira and colleagues [10] describe adaptive changes in the KoRV envelope gene associated with koala infection, highlighting the need for future functional comparisons between the mouse, gibbon, koala and any intermediate retroviruses in order to identify sequences correlated with exogenous and endogenous growth, and to determine whether adaptation to these alternative lifestyles has taken place. For example, one could speculate that selection for low levels of virus replication, perhaps as a result of a weak promoter, would favor virus persistence in the endogenous state but would be incompatible with the exogenous lifestyle.

Benign passenger or pathogen?

An integrated provirus can have five possible fates [2,11]: it can serve as a source for infectious virus; it can evolve to give rise to a viral genome that amplifies itself solely intracellularly; it can decay into junk DNA; it can undergo recombination between the LTRs to leave a solo LTR; or it might contribute a gene that can have a physiological function in the host [12]. These outcomes range from potentially harmful to beneficial to the host. Most replication-competent ERVs identified to date seem relatively nonpathogenic; a species harboring a lethal virus over an extended period of time would presumably be unlikely to survive unless it developed effective countermeasures to prevent virus replication [2,11]. One such measure would be to alter the normal cellular receptor for the virus in such as way as to prevent virus infection but not to affect the normal function of the cellular protein. This phenomenon is known as xenotropism, and explains why some species have multiple genomic copies of replication competent ERVs that can no longer infect cells from those species [13]. Retroviral evolution may also be influenced by the parallel evolution of antiviral factors such as APOBECs (which mutate or lead to the degradation of the products of reverse transcription) or Trim5 and Fv1, intracellular factors that bind to retroviral capsid protein, interfering with post entry events in the viral life cycle [14].

The size of each group of ERVs can vary significantly, presumably reflecting the ease and extent with which viral amplification took place following the initial germ-cell infection. Differential rates of ERV amplification may reflect the properties of the initial provirus. A virus that has undergone a debilitating mutation just before germ-cell infection is unlikely to give rise to many progeny, and studies of

proviruses artificially introduced into the germline by infection of pre-implantation embryos have shown that a surprisingly high percentage of novel proviruses carry such mutations [15]. Alternatively, an initial burst of amplification may be favored by simultaneous expression of endogenous and exogenous viruses. The koalas that have been examined so far have very high levels of circulating KoRV in the blood (viremia) [7], and it is not at all clear whether this results simply from reactivation of recently acquired germline proviruses or whether the animals have also been infected by exogenously transmitted KoRV. It will be important to determine whether germline KoRVs in viremic koalas are still being amplified from generation to generation, and if so, whether such an increase results from amplification of inherited endogenous provirus or from exogenously acquired virus. Similarly, it will be essential to follow the geographic spread of endogenous KoRVs into new locations and ask whether this is due simply to the interbreeding of infected and uninfected animals or whether there is spread of exogenous virus followed by new germline insertions.

The cross-species spread of retroviruses, generating novel ERVs, can be considered a natural evolutionary force. It remains to be seen whether KoRV will belong to the category of benign viruses or whether its presence will compromise the ability of koalas to survive. KoRV appears to be associated with the fatal lymphomas that kill many captive animals [7]. It may also be immunosuppressive, thereby contributing to the chlamydial infections that afflict many koalas [1,16]. The koala already faces the dual threat of shrinking habitat and inbreeding; will KoRV be one burden too many to bear? If so, should we be interfering, perhaps by vaccination, in an attempt to protect it from extinction? If the virus is spreading by exogenous infection followed by new germline insertions, it could be that an appropriate vaccination strategy might stop its spread. Any intervention may well entail laboratory studies, perhaps involving the deliberate infection of koalas. This would appear to be a case where use of some animals in research might be essential to the survival of their species. Hopefully, such studies will simultaneously prove informative about the elements making up a significant fraction of our genomes.

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