Chronicle / *Lab Book*

Neanderthals' Lasting Legacy

Early encounters with Neanderthals left marks on human genes.

NEANDERTHALS MAY HAVE died out tens of thousands of years ago, but they live on in our DNA. When early humans migrated out of Africa, they encountered, and mated with, some of their Neanderthal cousins. As a result, many people today have about 2 percent Neanderthal DNA in their genomes. David Reich, an HHMI investigator at Harvard Medical School, recently led a team that sleuthed which modern genes can be traced back to these ancient trysts.

The researchers compared DNA from a Neanderthal woman's remains discovered in Siberia to DNA from 1,004 present-day people. "The goal was to understand the biological impact of the gene flow between Neanderthals and modern humans," says Reich. "We reasoned that when these two groups met and mixed, some new traits would have been selected for and remained in the human genome, while some incompatibilities would have been selected against and removed."

Some of the DNA that endured the test of time left its mark on our hair and skin. Reich and his colleagues discovered that a number of the Neanderthal genes that exist in people today are involved in making keratin, a fibrous protein that lends toughness to skin, hair, and nails. Reich speculates that the Neanderthal versions of these genes may have helped humans adapt to non-African environments by producing thicker hair and skin to withstand a colder climate or to shield the humans from pathogens.

The study, published January 29, 2014, in *Nature*, also indicates that Neanderthals and early humans were "at the very edge of being biologically compatible," Reich says. The team found little Neanderthal DNA in the human X chromosome or in genes that are normally highly expressed in testes. This pattern is often linked to a phenomenon called hybrid sterility—when two organisms are distantly related, their male offspring can be rendered infertile. Thus, modern males who inherited a Neanderthal X chromosome may not have passed along that X chromosome to offspring.

Reich and his colleagues also discovered that some genes associated with a risk of lupus, diabetes, and Crohn's disease most likely originated in Neanderthals. The group's findings may help scientists glean more information about human disease genes, Reich says. – *Nicole Kresge*

About 2 percent of the human genome can be traced back to encounters with Neanderthals.

IN BRIEF

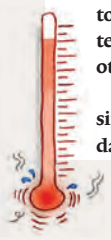
MALARIA HITS THE HIGHLANDS

When British colonists came to Africa in the nineteenth century, they would often seek refuge from heat and disease in "hill stations." These towns, built in the cool tropical highlands, were less likely to harbor the heat-loving mosquitoes that carry malaria. However, these and other high-altitude locations may soon be prone to malaria as well. According to HHMI Investigator Mercedes Pascual, climate change is increasing the risk of malaria transmission in these regions.

"There has been ongoing, heated debate on the role of climate change in the increased incidence of malaria observed from the 1970s to the 1990s in the East African highlands," explains Pascual of the University of Michigan. "One challenge has been to isolate the effect of a trend in temperatures from that of many other changing factors."

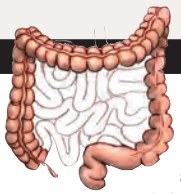
She and her colleagues sifted through malaria records dating to the 1980s from densely populated areas in the highlands of Ethiopia and Colombia, South America. They found that as temperatures increased, more cases of malaria at higher elevations occurred. When temperatures cooled, the disease retreated to lower elevations. Because the team focused on how malaria cases shift in altitude in response to yearly temperature changes, other variables that influence malaria trends, such as drug resistance and fluctuation in rainfall, were discounted.

The findings, reported March 7, 2014, in *Science*, underscore the need for sustained and increased intervention, including mosquito control, to mitigate the effect of climate change in these areas. Otherwise, Pascual and her colleagues have estimated, future temperature increases could result in millions of additional cases of malaria in Ethiopia alone.



A THERMOSTAT FOR MUCUS

Our guts are constantly exposed to bacteria, some helpful, some harmful. If gut defenses aren't strong when unfriendly bacteria attack, the pathogens can lead to increased susceptibility to diseases such as colon cancer and type 2 diabetes. Fortunately, humans and other animals have a thin lining of intestinal mucus that helps keep the bad guys at bay. Recently, three scientists—Richard Flavell, an HHMI investigator at Yale University, Eran Elinav at the Wiezmann Institute of Science, and Brett Finlay at the University of British Columbia—collaborated to show that this entire defense system depends on a protein complex called the NLRP6 inflammasome.



Inflammasomes are collections of proteins responsible for turning on immune responses that result in inflammation. In mice engineered to lack the NLRP6 inflammasome, no intestinal mucus shield was produced. Without that layer, the team reported on February 27, 2014, in *Cell*, bacteria began to attack the lining of the mouse gut, causing infection.

The scientists believe that the NLRP6 inflammasome acts as a thermostat that opens and closes the mucus faucet. The protein complex senses the amount of mucus needed and tells mucus-producing cells how much of the antimicrobial liquid to make. In mice without the NLRP6 inflammasome, the faucet stays closed and there is no mucus shield. This finding is surprising, Flavell explains, because "it was thought that the mucus layer was maintained in a constitutive fashion—in other words, it was essentially present at all times."

They next plan to test whether the inflammasome-mucus system works the same way in humans and to figure out how to "dial up" the protective shield.

CHANNEL CHECKPOINTS

Every second, more than one million calcium ions must squeeze through individual calcium channels into cardiac muscle cells to keep the heart

Photo: AquilaGib (Wikimedia Commons); Illustration: Leif Parsons

To watch the Cas9 complex in action, go to www.hhmi.org/bulletin/spring-2014.

CRISPR's Little Helper

Bacteria use a tiny signal motif to save time when detecting foreign DNA.

BACTERIA HAVE A secret weapon for dealing with viral invaders: a library of genetic mug shots. These bits of DNA, collected from previously encountered viruses, help the bacteria target and destroy their invaders. HHMI scientists recently showed that this defense mechanism—known as the CRISPR-Cas system—gets some of its accuracy and speed from a tiny sequence of DNA that is just three nucleotides long.

The workhorse of the CRISPR-Cas immune system is an enzyme called Cas9. Each Cas9 molecule carries a 20-base pair "guide" RNA sequence that matches one of the DNA mug shots. When a repeat offender invades, it's up to the Cas9 complex to find the complementary DNA sequence on the pathogen and to cut it.

Scientists also use the CRISPR system in their research labs to make precise changes in the genomes of animals and plants. "One of the concerns for people who are using this as a tool has been whether there are off-target effects, in which sites are mistakenly recognized by Cas9 and perhaps cut or modified in experiments," says HHMI Investigator Jennifer Doudna from the University of California, Berkeley.

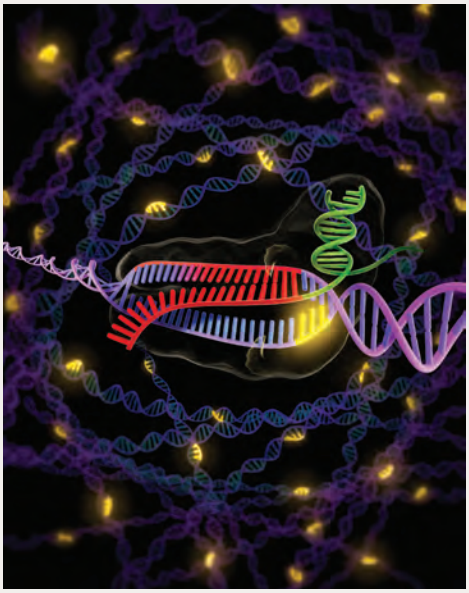
Doudna teamed up with HHMI Early Career Scientist Eric Greene of Columbia University to figure out how Cas9 does its job. Using a technique called a DNA curtains assay that was pioneered by Greene, the scientists were able to watch Cas9 interact with individual DNA molecules. Their findings were published on March 6, 2014, in *Nature*.

Instead of inspecting the entire genome of an invading virus, Cas9 bounces onto and off of the viral DNA while looking for a specific three-letter sequence of nucleotides, called a PAM. "The enzyme really only spends time at sites that have this motif," explains Doudna.

Cas9 unzips the DNA at each PAM site, looking for sequences that match its own guide RNA. There may be thousands of PAM sites, but only one sits next to the DNA pattern that Cas9 is looking for. If there's no match, Cas9 falls off, and the search continues.

Knowing that Cas9 relies on a PAM sequence in addition to its RNA guide molecule will help reduce concerns about the technique and will guide efforts to make Cas9 a better tool in genome engineering.

–Nicole Kresge



Short DNA sequences known as PAMs (yellow) enable bacteria to recognize and destroy foreign DNA.

Cas9 illustration: K. C. Roeyer; Chromosome illustration: Leif Parsons

beating, among other functions. This is an impressive feat—especially because sodium ions are the same size as calcium ions and are 70 times more plentiful outside the cells, yet few of them pass through the tiny pores. Using x-ray crystallography, HHMI Investigator Ning Zheng and his collaborators in William Catterall's group at the University of Washington recently caught a calcium channel in action and revealed the secret of its selectivity.

As they reported in *Nature* on January 2, 2014, the key to this filter is a series of three checkpoints that ions must pass through. Each of these three sites selectively binds to hydrated calcium and rejects other ions. The first site, near the mouth of the pore, recognizes calcium and admits it into the channel. Once inside, the calcium ion binds to the second site, where it remains until it's pushed out by the next calcium ion entering the channel. The final checkpoint, near the end of the channel, helps move the ion into the cell's interior.

Next, Zheng would like to figure out how drugs disrupt these calcium channels. "The mammalian voltage-gated calcium channels are the molecular targets of therapeutic drugs in the treatment of hypertension, angina pectoris, and cardiac arrhythmia," he explains. By understanding how the drugs interact with calcium channels, scientists can create new compounds that work better and have fewer side effects.

THE ROOTS OF A DISEASE

French Settlement, LA, is an unassuming little town just northwest of New Orleans. It is also the place where a group of genetic disorders known as hereditary spastic paraplegia (HSP), or French Settlement Disease, was first discovered. Unlike the town, the disease is anything but modest: its main features are stiffness and involuntary contractions in the legs. Thirty-five years after the disease's discovery, a study led by HHMI Investigator Joseph Gleeson at the University of California, San Diego, has almost doubled the number of genes associated with HSP.

Scientists had already linked 22 genes to HSP, but mutations in those genes explained less than 50 percent of the cases. To find more genes, Gleeson and a team of 51 scientists from around the world recruited 55 families with HSP. The scientists sequenced every gene in 93 family members and discovered 18 genes newly linked to the disease. They then created an "HSPome"—a genetic map showing how all the HSP-associated genes interact with each other.

The effort, which took 10 years, allowed the researchers to link HSP to other common neurodegenerative diseases, such as Alzheimer's. "This told us that common neurodegenerative diseases share similar networks and cellular vulnerabilities," says Gleeson. "Maybe we need to think about these less as individual diseases and more as collective problems of neuronal susceptibility."

The findings, reported January 31, 2014, in *Science*, may help Gleeson and his colleagues develop new treatments for HSP. They're already pursuing several promising targets.

DNA DOESN'T HAVE THE LAST WORD

During transcription, the RNA polymerase enzyme reads information from a strand of DNA and uses it to create RNA. It's often assumed that the RNA is an exact copy of its DNA template. Three years ago, Vivian Cheung, an HHMI investigator at the University of Michigan, showed otherwise. She found widespread sequence differences between RNA transcripts and their template DNA in human cells. In her latest research, Cheung has pinpointed when these mismatches, or RNA-DNA sequence differences (RDDs), occur.

Cheung and her collaborator, John Lis at Cornell University,

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Hidden Killers

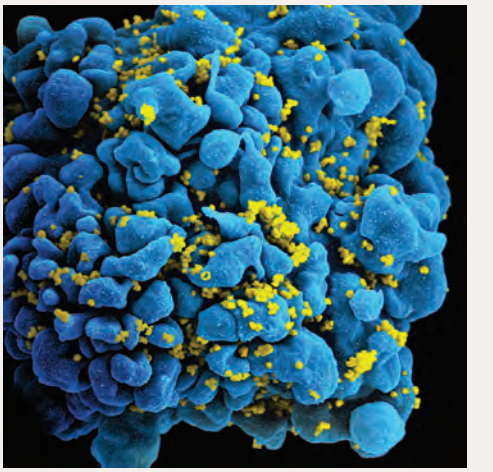
A larger-than-imagined reservoir of HIV is evading current antiretroviral therapies.

THERE WAS A time when a positive test for human immunodeficiency virus (HIV) meant certain death. Today, the future is much brighter for people with HIV, thanks to advances in drug treatments. But even the most powerful therapies can't flush out the dormant virus that lurks in a patient's body—a reservoir that, according to HHMI Investigator Robert Siliciano at the Johns Hopkins University, may be up to 60 times larger than previously imagined.

HIV wipes out the immune system by converting the very cells that are meant to help kill it into virus-producing factories. During an infection, HIV injects its genetic material into activated CD4+ T cells. The HIV DNA integrates into a T cell's genome, causing it to create more virus. Most of the infected T cells in this viral production mode die, but some survive and go into a resting state in which virus production is shut off. About 100 to 1,000 resting CD4+ T cells per million harbor dormant HIV sequences called proviruses. These sleeper viruses are invisible to circulating immune cells—and to current antiretroviral therapies.

Until recently, scientists were unable to get an accurate fix on the size of the provirus reservoir. Moreover, they didn't know how many of these stealthy invaders could wake up and mount an attack against the immune system. Siliciano and Ya-Chi Ho, an HHMI international student research fellow, published a technique in the October 24, 2013, issue of *Cell* that has revealed both the size and the composition of this reservoir. The good news is that about 88 percent of these dormant viruses have genetic defects that make them unable to reactivate. Unfortunately, the remaining 12 percent are fully functional and have the potential to reawaken and attack at any time. The findings suggest that previous calculations vastly underestimated the magnitude of the provirus population—by about 60-fold.

To effectively target HIV, future therapies need to consider these dormant viruses. "It doesn't mean that it's hopeless, but it does mean we need to focus on getting an even clearer idea of the scope of the problem," says Siliciano. Currently, he and his colleagues are working on simple clinical assays to assess reservoir size. – *Nicole Kresge*



HIV (yellow) attacks the immune system by infecting T cells like this one.

T cell: NIAID; Synapse illustration: Leif Parsons

IN BRIEF

isolated newly made RNA from human cells and compared the sequences to the DNA in the same cells. They discovered that RDDs are not formed when RNA polymerase creates an RNA copy of the DNA template. Instead, the changes often occur right after the synthesis, when the new RNA folds back on its DNA template, forming a DNA-RNA hybrid called an R-loop. These structures are thought to play a role in gene regulation. The group published its findings on March 13, 2014, in *Cell Reports*.

Now that Cheung knows when RDD formation occurs, she's looking for the processes that form these mismatches. "The knowledge that RDDs are not as rare as previously thought tells us that genetic studies need to give RNA variation some attention," she says. Figuring out the mechanisms behind RDD formation will further the understanding of how RNA processing contributes to genetic diversity.

REPROGRAMMING CANCER

Glioblastomas are the most common brain tumors. They are also the most lethal, in part because of a small population of stem cells that live inside each tumor. Although the stem cells comprise only a small portion of the tumor—just a few percent—they are not trivial. They cause aggressive tumor growth as well as resistance to radiation and chemotherapy. Recent findings by HHMI scientists point to a way to disarm these cells by focusing on what makes them so different from the rest of the tumor.

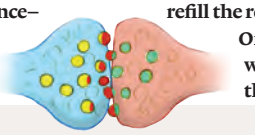
HHMI Early Career Scientists Bradley Bernstein, at Massachusetts General Hospital, and Aviv Regev, at Massachusetts Institute of Technology, collaborated to examine the circuits that regulate genes in both stem cells and non-stem cells from glioblastoma tumors. The researchers found four transcription factors—proteins that turn genes on and off—that were present only in the stem cells. When they expressed a cocktail of four transcription factors in the non-stem cells from the glioblastomas, those cells turned into stem cells. The experiments, published April 24, 2014, in *Cell*, show that transcription factors can override a glioblastoma cell's programming and drive it into a more aggressive state.

Bernstein and Regev hope to use this information to target these aggressive stem cells with small molecule inhibitors.

NEURONAL RECOVERY

In the body, neurons use molecule-filled sacs, called vesicles, to communicate. When it's time to send a message, a neuron's vesicles fuse with its membrane, releasing their contents into the synaptic space between the cells. If there is a lot to communicate, the neurons burn through their vesicles quickly.

Rapid replenishment of vesicles is of the utmost importance—the neurons need to be ready to send the next batch of signals.



HHMI Investigator Edwin Chapman and his team at the University of Wisconsin recently showed that two calcium-binding proteins, calmodulin and synaptotagmin 7, work together to ensure that neurons have adequate vesicles for communication.

One route of restoring synaptic vesicle supplies depends on calcium and calmodulin. Chapman's team discovered that, in response to Ca^{2+}, synaptotagmin 7 binds to calmodulin, and this complex initiates the vesicle replenishment pathway. The study, published in *eLife* on February 25, 2014, clarifies a number of controversies about the function of synaptotagmin 7 in the nervous system.

Now that Chapman knows what's involved in the pathway, he wants to figure out how the components work together to replenish the vesicles. "Do they clear out release sites so incoming vesicles can dock and fuse? Do they refill the releasable pool of vesicles? Or do they do both?" he wonders. "At the moment, this remains a mystery."