Palo Alto, California, lab. FIAU seemed a perfect fit. (After the FIAU disaster, a study in woodchucks infected with their own version of hepatitis B did detect the drug's toxicity, but woodchucks are rare in toxicology labs.)

In the new study, Peltz's team compared various doses of FIAU in the TK-NOG mice, using NOG mice that retained their mouse livers as controls. The first experiment, which gave the mice a high dose of FIAU, began on a Monday. Peltz thought it would take a few months before any toxicities showed up in the TK-NOG animals. "On Wednesday, [the lab] called and they said, 'You may want to stop this. The mice are really sick,' " Peltz remembers. "It was the happiest I've ever been about a sick mouse." One mouse had died and 14 others had acute liver failure. FIAU had no effect on the NOG mice. Lower doses of FIAU caused illness or serious liver abnormalities in the TK-NOG mice, and the severity of the effects increased with the dose—a key indication, the authors argue, that the drug caused the toxicities. Different doses of a safe, widely celebrated hepatitis C drug, sofosbuvir (*Science*, 13 December 2013, p. 1302), did not harm the TK-NOG mice.

Markus Grompe, a pediatrician at Oregon Health & Science University in Portland, says the new work is a "solid report" and "good proof of principle." But Grompe, who has a company that markets his own chimeric mice, says he expects all the existing mouse models with humanized livers, including his own, would have performed equally well. Peltz, who says "at present" he has "no financial interest" in his mice, insists that other chimeric mice have ongoing liver toxicities caused by the process used to engineer them; the two researchers slugged it out in the May and August 2013 issues of *Trends in Pharmacological Sciences*.

Charles Rice, who studies hepatitis C virus at Rockefeller University in New York City, calls Peltz's study "interesting" but says "critical controls" are missing. He says the immunodeficiency in the TK-NOG mice could have contributed to FIAU's effects. To rule this out, Rice says he'd like to see FIAU tested in TK-NOG mice reconstituted with liver cells from rats, dogs, and monkeys.

Peltz says the team will test other drugs that, like FIAU, did not show toxicity in animal studies but caused harm in humans. He'd also like to create chimeric mice with human liver cells from different genetic backgrounds to see if results from the mice could help link drug effects to specific populations.

Hoofnagle says the humanized mice might help answer a fundamental question about FIAU: Why did it cause harm in the first place? The drug exists in two different structures, or isomers, and some evidence suggests only one is dangerous to humans. Says Hoofnagle: "This model might be an elegant means to sort out these issues."

–JON COHEN

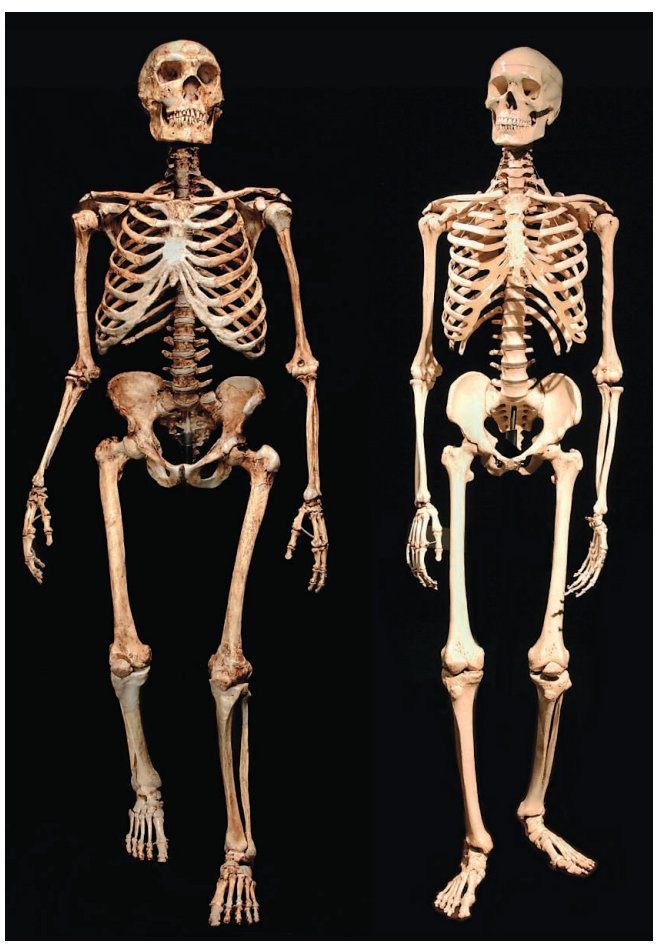
EPIGENETICS

Ancient DNA Holds Clues to Gene Activity in Extinct Humans

Ancient DNA researchers are teasing yet more secrets from long-vanished human species. Over the past 2 years, they revolutionized the study of human evolution by deriving high-quality genome sequences from 50,000-year-old fossils. Now, through clever analysis of that ancient DNA, they have gleaned a record of which genes were turned on or off in those archaic humans.

Online this week in *Science* (http://scim.ag/DGokhman), an Israel-based team, working with an ancient DNA research group, reports mapping the chemical modifications likely to have silenced genes in the DNA of a 50,000-year-old female from a mysterious group called the Denisovans (*Science*, 31 August 2012, p. 1028) and a slightly older female Neandertal. By comparing that data with similar maps for modern human tissue, they have pinpointed two genes that might underlie skeletal differences between us and our extinct cousins.

One or two genomes aren't enough to verify that the observed chemical modifications, known as methylation, are characteristic of the entire species, and much more work is required to confirm that the methylation patterns actually led to skeletal changes in Neandertals and Denisovans. Still, to be able to study gene activity at all in ancient humans is "ingenious," says Chris Stringer, a paleoanthropologist at the Natural History Museum in London.



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Boning up. DNA methylation that silences genes may account for some of the skeletal differences between Neandertals (*left*) and modern humans.

"This study shows that it is now possible to 'paint' ancient genome sequences with an additional layer of information," adds Ryan Lister, a genome biologist at the University of Western Australia, Crawley.

The past decade has driven home the importance of how genes are regulated, not just which proteins they code for, in defining how an organism looks, acts, and reproduces. In one key regulatory process, cells attach a methyl side group to a DNA base, typically cytosine. Methylation helps curtail the activity of rogue mobile DNA

and in mammals inactivates one of the X chromosomes in females. But it can also silence individual genes, for example when a gene has done its job and is no longer needed. And because methyl groups can be removed, genes can be called out of retirement at any time.

Lately, molecular and cell biologists have been mapping methylation patterns—so-called methylomes—in various tissue from people to learn what role this regulation plays in health and disease. But the method for mapping methylomes in living cells requires a lot of DNA, typically more than can be found in a fossil.

Adrian Briggs recently hit upon a possible solution to generating ancient methylomes. As a graduate student at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, Briggs worked in Svante Pääbo's lab, which is famous for sequencing ancient DNA. Over time fossil DNA degrades, breaking into fragments and changing chemically. The base cytosine sometimes transforms into a different base found in RNA, uracil, which genome sequencing machines misread as the base thymine.

While developing a way to delete the uracils, Briggs became confused, because the resulting sequence still had more thymine bases than expected. Then a biochemist colleague explained that a methylated cytosine would not degrade into uracil and instead would over time convert to thymine, resulting in an unusually high proportion of thymine in the sequence. A light bulb went off.

Briggs realized that by comparing an ancient DNA sample with a modern human genome, researchers could pinpoint places where the former had thymine but the latter has cytosine. A few of those differences might be actual mutations distinguishing the two species, but most would indicate where a cytosine had converted to a thymine, and thus, where methylation had occurred. "Normally DNA damage gives us a hard time," notes Ludovic Orlando, a developmental biologist at the University of Copenhagen. "For once, DNA damage becomes really our friend!"

> ***"This paper gives us a first glimpse of how we can begin to unlock epigenetic aspects of ancient genomes which have been hidden from us up to now."***
>
> **—CHRIS STRINGER, NATURAL HISTORY MUSEUM, LONDON**

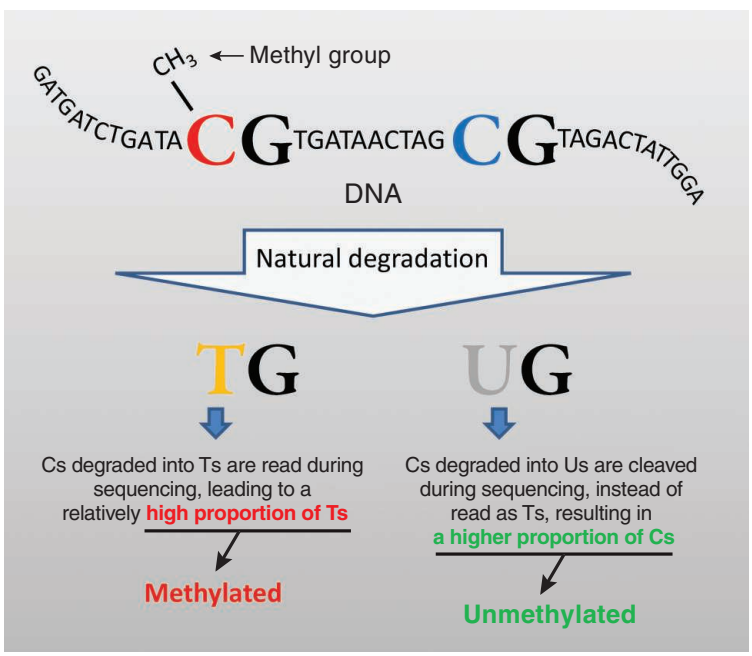
Briggs, who is now at a biotech company called AbVitro Inc. in Boston, published his idea in 2010, but no one could try it out because it required in-depth sequencing of a genome, something that was still out of reach for fossil DNA. But last year, with improvements in DNA sequencing, Orlando and colleagues tried a similar approach on a 4000-year-old Eskimo hair, describing the hair's methylome in *Genome Research* in December 2013. Because methylation patterns vary with age, they were also able to estimate how old the Eskimo was at death.

Then David Gokhman, Eran Meshorer, and Liran Carmel of the Hebrew University of Jerusalem teamed up with Pääbo's group to map methylation across a Denisovan and a Neandertal genome, both of which had been sequenced in depth—the former came from DNA in a fossil finger bone, the latter from a toe bone. When they compared the ancient methylation patterns with ones determined in bones from a modern young female and in other tissues, they found that about 99% of ancient and modern methylome maps match up. The match was perfect at housekeeping genes, which are essential to all cells and therefore less likely to have their regulation changed over the course of human evolution—an indication that the analysis was on target. "I was very convinced," says Lluis Quintana-Murci, a population geneticist at the Institut Pasteur in Paris.

At about 2000 places, though, the ancient and modern methylomes diverged. In both of the archaic methylomes but not the modern human one, there were signs of heavy methylation that could have silenced two of a cluster of genes critical to development, known as HOXD. The few studies that have looked at diminished HOXD gene function in mammals have found skeletal changes that approximate some of the features that distinguish Neandertals from modern humans, such as larger ends of the thigh bone, bigger hands and fingers, shorter limbs, and broader elbow and knee joints. Still, Orlando acknowledges, "There's a long way from spotting the differentially methylated region and saying this is what caused the differences [in anatomy]."

While Andrew Feinberg, a genomicist and epigenetics expert at Johns Hopkins University in Baltimore, Maryland, applauds the approach, he and others remain wary. "I don't think [the method] has been tested fully and I don't think they can draw inferences from a single sample," he says. He and others emphasize that because methylomes can vary through time, across environments, from cell type to cell type, and even between the sexes, researchers will need many more high-quality archaic genomes to establish the typical methylation pattern of Neandertals, Denisovans, or any ancient human species.

Nevertheless, many ancient DNA specialists are optimistic that such sequences will be forthcoming. And Briggs predicts an early application for the technique: Biologists interested in the changes in gene activity that helped transform wild animals into domesticated breeds will soon try the ancient methylome approach on livestock fossils. "This paper gives us a first glimpse of how we can begin to unlock epigenetic aspects of ancient genomes which have been hidden from us up to now," Stringer concludes. **–ELIZABETH PENNISI**



Methylation revelation. DNA bases covered with methyl groups degrade differently from unmethylated ones, leaving telltale signs in ancient genomes.

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