For decades scientists have known that at least 98% of human DNA is identical to that of chimpanzees. Now they have at last begun to explore which genes separate us from the apes

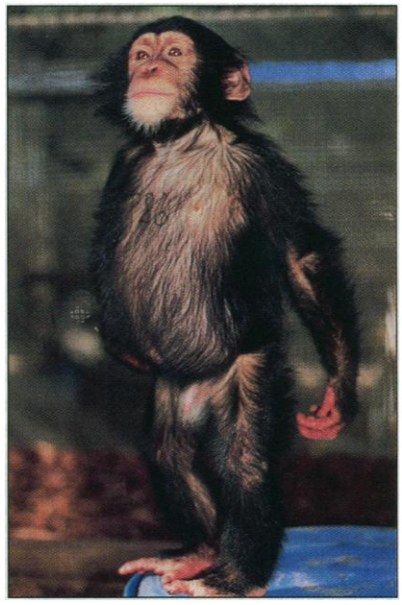
Which of Our Genes Make Us Human?

We humans like to think of ourselves as special, set apart from the rest of the animal kingdom by our ability to talk, write, build complex structures, and make moral distinctions. But when it comes to genes, humans are so similar to the two species of chimpanzee that physiologist Jared Diamond has called us "the third chimpanzee." A quarter-century of genetic studies has consistently found that for any given region of the genome, humans and chimpanzees share at least 98.5% of their DNA. This means that a very small portion of human DNA is responsible for the traits that make us human, and that a handful of genes somehow confer everything from an upright gait to the ability to recite poetry and compose music.

But what are these genes, so few in number yet so powerful in effect? Until now there has been little funding or research to track them down, and the primate genome has been almost virgin territory. "You could write everything we knew about the genetic differences in a one-sentence article," quips neuroscientist Thomas Insel, director of the Yerkes Regional Primate Research Center of Emory University in Atlanta.

Now that is changing, as a persistent band of geneticists and evolutionary biologists launches new studies to find differences in the genes, chromosomes, and biochemistry of humans and chimpanzees. Next month, researchers will report finding the first significant biochemical variation between humans and other apes: Humans lack a particular form of a ubiquitous cell surface molecule found in all other apes. Other teams are reporting newfound differences in the arrangements of DNA on the chromosomes of humans and other primates.

And new sequencing projects are starting to compare primate and human DNA base by base. Two new centers—at Yerkes and in Leipzig—opened simultaneously last year to study the molecular evolution of the great apes; next month, at an international meeting in Chicago, researchers will push for an organized, international primate genome project (see sidebar). Because primates are less susceptible than humans to certain diseases, including cancer and AIDS, the sequence differences are of more than evolutionary interest and are already drawing commercial attention. One Denver, Colorado, biotech company has already submitted patents on key human and chimp genes.



E. FERORELLI/YERKES REGIONAL PRIMATE RESEARCH CENTER

Aping it. Chimpanzees may adopt the occasional two-legged pose, but they differ dramatically from humans in anatomy and behavior.

For the moment, no one can tie the few known molecular variations with the familiar litany of chimp-human differences, such as body hair, language, or brain size. But the leaders of genomic and evolutionary research alike say that now is the time to explore those links. "This is one of the major questions that those of us interested in our own biology would like to ask—what does that 1.5% difference look like?" says Francis Collins, director of the National Human Genome Research Institute.

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Vive la difference

That question was first raised in print in a landmark 1975 paper by geneticist Mary-Claire King and the late biochemist Allan Wilson, both then of the University of California, Berkeley. They surveyed protein and nucleic acid studies and found that the average human protein was more than 99% identical to its chimpanzee counterpart; the coarse DNA hybridization methods of the time showed that the average nucleic acid sequence was almost as similar (*Science*, 11 April 1975, p. 107). Thus, King and Wilson concluded, humans and chimpanzees were genetically as similar as sibling species of other organisms, such as fruit flies or mice.

This left a great paradox: Our DNA is almost identical to that of our chimp cousins, but we don't look or act alike. "The molecular similarity between chimpanzees and humans is extraordinary because they differ far more than many other sibling species in anatomy and way of life," the pair wrote.

What's more, much of the DNA in any organism is so-called "junk DNA" that has no apparent function, and mutations in these regions do not change the function of genes. Thus, many of the genetic differences between humans and chimps probably don't affect the organisms at all. The challenge is to find those few mutations that do make a difference—either by altering genes that code for proteins or by changing how genes are regulated, King and Wilson said.

But although many labs have since confirmed that our nuclear DNA is 98% to 99% identical to that of chimpanzees, few have taken on the quest to find the differences that matter. "It was one of those fields that fell through the cracks," says Ajit Varki, a glycobiologist at the University of California, San Diego (UCSD), who has recently surveyed the known differences be-

Pushing a Primate Genome Project

Paleoanthropologists have long mined the stones and bones left by ancient humans for evidence of our past. But locked in the DNA of apes in zoos and tropical forests is an untapped treasure trove of clues about how we became human. "All sorts of attention is lavished on every new early human fossil out of Africa," says Edwin McConkey, a molecular biologist at the University of Colorado, Boulder. "But chimpanzees are astonishingly close to us genetically. Isn't it time to study these living links as well?"

McConkey is one of the leaders of a push for an international program to sequence DNA from chimpanzees and other great apes. The effort would supplement existing searches for the key molecular differences that set humans apart from our primate relatives (see main text). And now is the time to start, McConkey and molecular evolutionist Morris Goodman of Wayne State University in Detroit argued in a recent article in *Trends in Genetics*. Next month in Chicago, at a meeting organized by Goodman, a group of influential researchers will make the case for such a project, pointing to the current ability to scan genomes quickly and the ballooning amount of human genetic data. "All the technology is in place now," says Mary-Claire King, a geneticist at the University of Washington, Seattle.

Genome leaders say they are on board, although in the United States no one is talking about funding anywhere near the scale of the $1.5 billion Human Genome Project. "The timing is right for some pilot projects" to lay the groundwork for a complete primate genome project perhaps 5 years from now, says Francis Collins, director of the National Human Genome Research Institute (NHGRI). But while American scientists are talking about a project, work is already under way in Germany, where the German human genome project has awarded $1.1 million to the newly formed Max Planck Institute for Evolutionary Studies in Leipzig for comparative studies of human and primate genomes. They've already started with an effort to sequence comparable segments of DNA from six chromosomes in humans and chimpanzees, says Svante Pääbo, a molecular geneticist at the University of Munich who heads the work. "I think the majority of primate comparative genomics in the next few years is going to be done in Germany at Leipzig," says McConkey.

Other groups are gearing up in the United States, including one at Yerkes Regional Primate Research Center in Atlanta, where researchers opened a Living Links Center with $250,000 from Emory University to compare chimpanzees and humans using genetic, neuroanatomical, behavioral, and cognitive approaches. No sequencing is under way yet, but the center is negotiating an agreement with a Denver biotech firm, GenoPlex Inc., to do high-volume DNA screening. "What's strange is that several people have had the same idea at exactly the same time," says Thomas Insel, director of Yerkes.

So far, researchers have done little more than prove the worth of their tools and affirm the overall similarity of the human and chimp genomes. But the pilot projects are promising. For example, Collins's team at NHGRI tested DNA chips—a DNA-scanning technology made by Affymetrix of Santa Clara, California—to compare the sequence of a 3400–base pair segment of the human breast cancer gene *BRCA1* with the same gene in chimpanzees, gorillas, and orangutans. Using a large segment of human DNA as a reference on the chip, the researchers searched for sequence variations in the same gene in other apes. The technology was remarkably fast and accurate, Collins's group reported in February in *Nature Genetics*. "The DNA chip worked very well," says Collins. "It's a way to look at primate sequences faster than sequencing each primate's DNA de novo" and should keep down the cost of a full-scale primate genome effort once DNA chips become more affordable.

But chips aren't the only candidate technology, says Collins: "Other people are playing with gel-based methods and mass spectrometry." When the methods are refined and more affordable in 5 years or so, he predicts that human genome sequencing will have yielded a list of hot genes to study in primates, such as those that help equip humans for language, higher order brain function, and upright walking. Says King: "It's the next logical step of the Human Genome Project." **–A.G.**

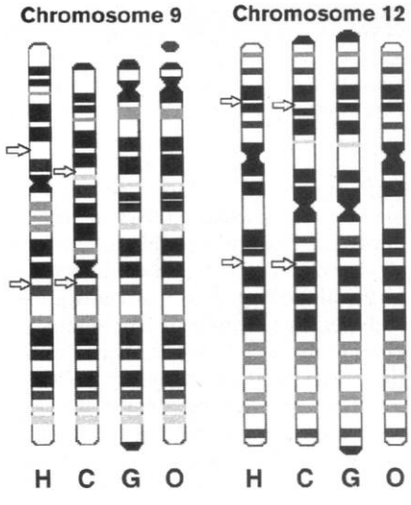
tween humans and apes.

The tools and funds for sequencing large amounts of DNA rapidly weren't available until recently. And this line of work required a bold shift in thinking for most labs. "Most people in comparative genetics ask what's similar and conserved," says molecular evolutionist Caro-Beth Stewart at the State University of New York, Albany, who trained with Wilson. "Just a few of us have been trying to ask: What's different? What makes us human?"

YUNIS AND PRAKASH, 1982

The biochemical trail

One way to answer that question is to start with biochemical differences, and then trace them back to their genetic origins. That approach has yielded its first big payoff, to be reported in the October issue of the *American Journal of Physical Anthropology*. After studying tissues and blood samples from the great apes and 60 humans from diverse ethnic groups, Varki and his colleagues Elaine Muchmore and Sandra Diaz at UCSD were surprised to find that human cells are missing a particular form of sialic acid, a type of sugar, found in all other mammals studied so far, including the great apes. "Now you've got something that is changing the surfaces of all cells in the body," says Varki.



Seeing the difference. Banding patterns on chromosomes 9 and 12 are clearly different in humans (H) and our ape cousins, the chimpanzees (C), gorillas (G), and orangutans (O).

The sialic acid molecule is found on the surface of every cell in the body, and previous work has shown that it can take on a surprising variety of roles. In some cases, it acts as a receptor for messages from other cells, but pathogens including those that cause cholera, influenza, and malaria also use it to gain a foothold on the cell. Chimpanzees are not as susceptible as humans to some of these pathogens, and the researchers speculate that this molecular change may be part of the reason why. There are even hints that sialic acid may be involved in cellular communication during brain development and function, says Varki.

The chimp and mammalian form of sialic acid, known as *N*-glycolyl-neuraminic acid (Neu5Gc), is modified from the basic form of the compound (called Neu5Ac) by the addition of an oxygen atom. But the human form is simply the basic acid, lacking the additional oxygen atom. That changes the shape of the molecule in a region that could alter how it is recognized by other molecules, whether pathogens or cellular

messengers, says Varki. Other researchers are intrigued: "I think this is really nice work," says Svante Pääbo, a molecular geneticist at the University of Munich and leader of a German effort to sequence ape genomes. "It's really the first difference [in the expression of a gene product] that has come up."

In recent months, Varki's team has traced this difference back to a gene that codes for a hydroxylase enzyme in apes, which adds the extra oxygen atom. Humans are missing a 92–base pair section of this gene, according to new results from a team of Japanese researchers, led by glycobiologists Akemi Suzuki and Yasunori Kozutsumi at Tokyo Metropolitan Institute. Varki's lab has a paper in press in the *Proceedings of the National Academy of Sciences* that includes similar studies on the gene in the great apes; they are now also working on human fossils with Pääbo's lab to see if this change was recent in humans.

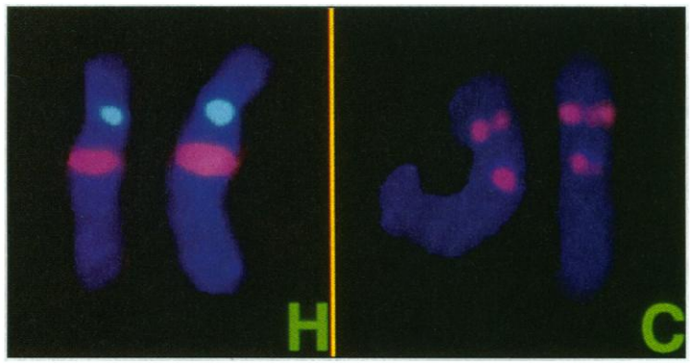
Yet the question still remains: Does this biochemical difference matter? No one has yet identified a specific function altered by the loss of this particular version of the molecule. "Until we know what this gene does, I remain skeptical of its importance," says Harvard University molecular anthropologist Maryellen Ruvolo. In search of clues, Kozutsumi is raising mice where the hydroxylase enzyme gene is knocked out, as it is in humans, to see if they produce the simpler form of sialic acid seen in humans—and if they have any anatomical or behavioral differences. "Maybe their mice will speak," jokes Varki, whose own lab is raising transgenic mice that overexpress hydroxylase in the brain to see if it affects anatomy or behavior.

But Ruvolo doesn't expect dramatic results: "Somehow, I can't believe that switching off the expression of this gene in adult humans is responsible for a myriad of important changes in human evolution." Most researchers, including Varki, agree that the difference won't come down to a single genetic change. "There won't be one magic gene that makes us human," says King, now at the University of Washington, Seattle.

Remodeling chromosomes

Another tactic for finding the key differences is to start with chromosomes, because even a simple karyotype—a picture of the chromosomes—reveals that other apes have 24 pairs of chromosomes while humans have 23 pairs. While 18 of the 23 pairs are virtually identical in humans and other apes, it has long been known that the remaining pairs have segments that have been reshuffled since the great apes went their separate ways (*Science*, 19 March 1982, p. 1525). In recent years, several labs in the United States and Germany have been homing in on this chromosomal remodeling.

For example, the gene mutated in a rare human disorder called adrenoleukodystrophy—made famous by the movie *Lorenzo's Oil*—turns up on the X chromosome in chimpanzees and humans. But nonfunctioning copies of it also have been found in different places in the chimpanzee and human genomes (*Science*, 12 June, p. 1693). Chromosomes 4, 9, and 12 have also been remodeled differently in chimps and humans, according to work published last month in *Genomics* by human geneticists David Nelson and Elizabeth Nickerson of Baylor College of Medicine in Houston. For example, the researchers spotted a chunk of DNA that sits on chromosome 4 in all apes and humans, but in chimpanzees it has moved to a new spot on the same chromosome and been inverted. The translocated chunk includes a gene called *AF4*, which codes for a transcription factor—and is mutated in some forms of acute leukemia in humans. Because apes are much less prone to certain cancers, including leukemia, this is an intriguing finding, raising the possibility that the inversion alters the factor's expression in chimps and so helps protect them from leukemia, says Nelson.



Genetic shuffle. On human chromosome 4 (*left*), the centromere is stained green, and a chunk of DNA is pink; on the chimp chromosome (*right*), the human centromere is lacking and the chunk of DNA has split into three pieces and moved.

ELIZABETH NICKERSON AND DAVID L. NELSON / BAYLOR COLLEGE OF MEDICINE

Still, the functional significance of this and other chromosomal differences between humans and other apes is unknown. One possibility, says Nelson, is that similar remodeling disrupted specific genes in our primate ancestors, altering human physiology or function. Because sperm and eggs can't mingle their genetic material unless the chromosomes line up properly, he adds that such rearrangements could have created a reproductive barrier between our ancestors and other primates—the first step in creating new species like our own.

But others, such as Pääbo, think that chromosomal rearrangements at influential sites are rare and so are skeptical that they play a major role in the differences between chimp and human. Pääbo and King think instead that the most promising research avenue is to identify small sequence differences that subtly change the expression of genes that regulate the timing of development, such as those that code for transcription factors that might lengthen the growth period of the brain and, hence, allow more complex brain structure in human fetuses.

The sequencing efforts that may reveal these differences are now under way. Pääbo's group in Munich and Leipzig has sequenced a 10,156–base pair segment of DNA in the X chromosome of humans and chimpanzees, confirming again that they are about 99% similar. Now they're seeking differences in the expression of the identified genes in the brain and in the immune system.

And at GenoPlex Inc., a Denver-based company founded last year by University of Colorado Health Sciences Center geneticists Jim Sikela and Tom Johnson, researchers have come up with a rapid method to find meaningful sequencing differences between humans and chimps. After sequencing a stretch of DNA in each species, they count two different types of nucleotide differences: those that change the structure and function of a protein product, and silent substitutions that don't. If the ratio of replacement to silent substitutions is high, they consider that the gene sequence is likely to have undergone a functional change that was selected for in humans.

Preliminary results suggest that they have found uniquely human genes involved in AIDS susceptibility and learning and memory, says Walter Messier, an evolutionary biologist at the company. The firm has submitted patents on novel uses of these gene sequences, which they hope may become targets for new drugs.

Much of this work is in its infancy, but researchers say they are poised on the verge of a brave new world where they will be able to identify and tinker with the DNA that makes us human—and will face new ethical dilemmas. "What happens if scientists identify a human gene that controls development of the larynx—a gene that might give chimpanzees the anatomy needed for speech?" asks Edwin McConkey, a molecular biologist at the University of Colorado, Boulder. "Can you imagine the ethical debate involved in whether or not to create transgenic chimps? It will open a real Pandora's box." **–ANN GIBBONS**