Chromosomes: The Ends in View

Textbook illustrations aside, no one has really known what caps the human chromosomes or where, in fact, they end. Now they do, with the apparent isolation of a human telomere

No one knows what, exactly, is at the tips of human chromosomes, or where, for that matter, the chromosomes actually end. Since the early 1970s it has been clear that the ends of chromosomes—the telomeres—must be different from the middle, otherwise the chromosomes would be whittled away with each successive round of DNA replication. But the nature of the telomeres—indeed, their very identity—has proved hard to pin down.

Now Robert Moyzis and his colleagues at Los Alamos National Laboratory think they have found the long-sought human telomere, the piece of DNA that caps both ends of all 23 chromosomes. It is a sequence of six nucleotides, repeated over and over again, without a mistake. And if recent findings in protozoa are any indication, the telomere will be found to twist itself into novel configurations and form guanine-guanine bonds that defy the rules of DNA-binding laid out by Watson and Crick.

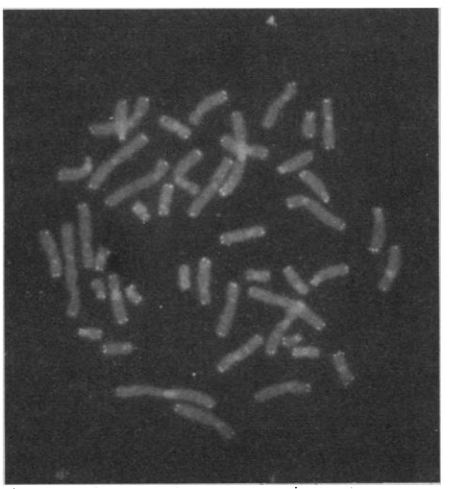
The evidence is not airtight, but it is certainly compelling. "The sequence is right at the end of the chromosome, as best we can determine," says Moyzis, who announced the finding at a recent meeting at Cold Spring Harbor Laboratory.* It will be published in an upcoming issue of the *Proceedings of the National Academy of Sciences.*

This new work follows close behind startling new findings about how telomeres work in lower eukaryotes, as well as the first isolation, in April, of a telomere from a higher eukaryote, the flowering plant *Arabidopsis thaliana.* And now, apparently, the human telomere has been isolated.

The implications are many. First, the sequence Moyzis and his colleagues have found is an immediate godsend for those investigators constructing genetic maps of the various chromosomes.

"For the first time we have an end to the map," says Ray White of the Howard Hughes Medical Institute at the University of Utah, who heads one of two groups working on a genetic linkage map of the entire human genome. The problem in genetic mapping has been knowing when to stop: if you don't know where the end is, then you clearly don't know how far to go. It's a bit like mapping the United States but leaving off California.

Chromosome 10 has proved particularly vexing, says White. "We have been feeling our way down the chromosome, blind. Each time we think we have the end nailed down we find another marker that adds another 60 centiMorgans [a measure of genetic distance]. The telomere is crucial to our sense of completion. It is very helpful to know when to stop."



Telomeres. *The new-found sequence shows up at the tips of all the human chromosomes.*

This new sequence also provides a way to get at the DNA out near the ends of the chromosomes, which, for some reason, is where much of the genetic action is. The immediate benefit will be to accelerate the hunt for disease genes located out near the tips, such as the Huntington's gene on chromosome 4 and the polycystic kidney disease gene on chromosome 16, which have been notoriously hard to find.

And, says Maynard Olson of Washington University, the telomere is "an entry point to get probes that are specific for each chromosome end," and there are 47 distinct chromosomal ends. Comments Olson: "This will obviously take some work."

Olson and Moyzis are already collaborating on a strategy to clone, in yeast artificial chromosomes, the DNA that nestles up against the human telomeres—DNA that promises to be tricky to clone. "If this approach works," says Moyzis, "it should be possible to get directly at any particular end you want. All the adjacent sequences will be interesting. The recombination frequency goes up a lot near the end, so it seems like a dangerous place to put genes. It will be intriguing to see what humans put there."

Perhaps the greatest boon, at least to Moyzis, is the insight this discovery will yield into how the telomeres work and the role they play in chromosome organization and function. While geneticists have made impressive strides in identifying genes and charting their location on chromosomes, fundamental questions about the chromosome as a whole—in essence, what makes a chromosome more than just a string of genes—are just now being addressed.

Much of what is known about telomeres comes from work by Elizabeth Blackburn and her colleagues at the University of California, Berkeley, on *Tetrahymena*, a ciliated protozoan with a small genome and an abundance of telomeres. Ten years ago Blackburn and Joseph Gall found that the *Tetrahymena* telomere consists of no more than tandem repeats of a short DNA sequence. Since then, all other telomeres isolated from lower eukaryotes have fit the same pattern: a short, repeated sequence, with one DNA strand rich in guanine; the other in cytosine.

With the isolation of the human telomere, in addition to the *Arabidopsis* telomere a month ago, it looks as if all eukaryotic telomeres are surprisingly alike, says Moyzis, and equally ingenious.

Until now, despite attempts by numerous labs, the human telomere had escaped detection. The Los Alamos group succeeded through a novel approach to ensure that the DNA they were scouring was rich in repetitive sequences. Once dismissed as "junk," repetitive DNA is now thought by many to be vital to chromosome structure and function. Long blocks of repetitive sequences, for example, have been found around the human centromere—and some suspect they may be the centromere itself. Similarly, Moyzis reasoned, "if the same sequence is at the end of each chromosome, then it, too, is likely to

*"Genome Mapping and Sequencing," 27 April to 1 May.

be a repetitive sequence," as Blackburn's work had shown.

The problem, however, is that tandemly repeated sequences are notoriously underrepresented in human chromosome libraries, the collections of DNA that are the usual starting place for genetic analyses. To construct a library that would contain these sequences, Moyzis fragmented the DNA physically, by "shoving it through a narrow gauge needle," rather than by relying on restriction enzymes to snip the DNA. Restriction enzymes cut at particular sequences, or cutting sites, which, Moyzis reasoned, are likely to be few and far between, if they occur at all, in blocks of repetitive DNA.

"In retrospect," says Moyzis, "it was a very good decision." Not only did the library contain repetitive sequences, but six of the clones were nearly identical in rodent and humans. Such evolutionary conservation suggested that they must be vital for cell function.

Upon sequencing, two of the clones turned out to be pure tandem repeats of six nucleotides, TTAGGG, repeated over and over again, without a single variation—something the group had never encountered before. Moreover, the sequence was identical to the telomere of trypanosomes, infectious microorganisms, that had been sequenced several years earlier by Blackburn.

Chances are, the group realized, that this sequence was the human telomere as well. If so, then it should be present in roughly the same amount on each chromosome regardless of chromosome size, in contrast to other repetitive sequences, which show up in larger amounts on the larger chromosomes. It was. What's more, every chromosome carried it at both ends, in repeats ranging from 1500 to 6000 nucleotides in length per tip.

The key experiment, says Moyzis, was to use an enzyme, Bal 31, that chews DNA molecules sequentially from each end. Doing so verified that the repeated sequence resides right at the physical end of the chromosome, and not several thousand bases in. "There could be ten nucleotides or so more of something else further out, but the sequence is at the extreme end of the chromosome," says Moyzis. "It is likely to be the real telomere."

The identical sequence shows up at the chromosome tips of a wide range of mammals, birds, and reptiles, including the Indian muntjac, or barking deer, and the Japanese raccoon dog, as well as the more ordinary chimpanzees, orangutans, and hamsters. "We see the sequence at the telomere in everything we have looked at down to reptiles, despite the size of their chromosomes and their number," says Moyzis, who is now looking at nematode and *Drosophila* to get a fix on when the sequence evolved as the human telomere. It is also remarkably similar—varying in just one nucleotide—to the *Arabidopsis* telomere, recently isolated by Eric J. Richards and Frederick M. Ausubel of Harvard.

Evolutionary conservation

The Indian muntjac, or barking deer, has the exact same telomere at the ends of its chromosomes as do humans and a variety of other mammals and birds and reptiles.

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This striking evolutionary conservation, as well as the sequence's terminal location and its close resemblance to functional telomeres isolated from lower eukaryotes, suggest that this is, indeed, the human telomere. Moyzis cautions, however, that definitive proof awaits a demonstration that this sequence functions as a telomere.

Finding out will involve seeing how this putative telomere finesses the replication problem. As mentioned earlier, without a special strategy, linear chromosomes would be progressively shortened during each round of DNA replication.

In her extensive analysis of *Tetrahymena*, Blackburn and her colleagues recently found how the telomeres do this, at least in lower eukaryotes. A previously unknown enzyme, which her group dubbed "telomerase," synthesizes the ends of chromosomes de novo; adding the telomeric sequences, in a nontemplated way, back on to the chromosome one base at a time.

Now the question is whether human chromosomes add telomeres enzymatically, too, as Moyzis suspects they do. Such experiments are just beginning.

And in the past few months Blackburn and Ignacio Tinoco made the surprising finding that the telomere is capable of novel DNA-DNA interactions, in which one strand twists itself into a hairpin configuration, anchored by guanine-guanine bonds instead of the usual guanine-cytosine bonds in the rest of the double helix.

This structure, Blackburn suspects, is intimately involved in telomere replication. If so, this would be the first evidence that a nonstandard configuration, a non–Watson-Crick base-paired DNA configuration, could play a fundamental biological role.

Moyzis suspects that the human telomere, too, will have these unique physical properties and probably more. To find out he has sent the sequence to Alexander Rich at the Massachusetts Institute of Technology to determine, through x-ray crystallography, just what it does.

Whether the hairpin structure—or the sequence itself—is involved in chromosome stability, the other mysterious telomere function, remains to be seen. Without something unique at its ends, the chromosome would be unstable—the ends would fuse or the endmost nucleotides would be knocked off—as happens when a chromosome is fragmented, say, if it is zapped with an x-ray. Somehow, the telomeres send a signal to the cell saying: "I am a separate entity, keep me intact."

"In lower eukaryotes the data are increasingly clear that it is this sequence that does both," says Moyzis. "Now that we have got the sequence out of humans, we can test it."

And if this does, in fact, turn out to be the telomere, it may be possible to begin constructing artificial human chromosomes, similar to the yeast artificial chromosomes that have proved so useful in cloning and gene transfer. Artificial human chromosomes, or mini-chromosomes, would open up new possibilities for studying chromosome function, as well as new techniques for gene transfer and, much more speculatively, gene therapy. And, says Moyzis, the ultimate proof that this is the telomere will be whether it functions as one in an artificial chromosome.

As was the case with yeast, success in building an artificial chromosome hinges on having the key chromosomal elements—the telomere and the centromere (and for yeast, the replication origin as well). The telomere, apparently, has been found, and Moyzis, as well as several other investigators, are now "hot on the trail of the centromere." ■

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