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Visualizing the End of the Human Genome

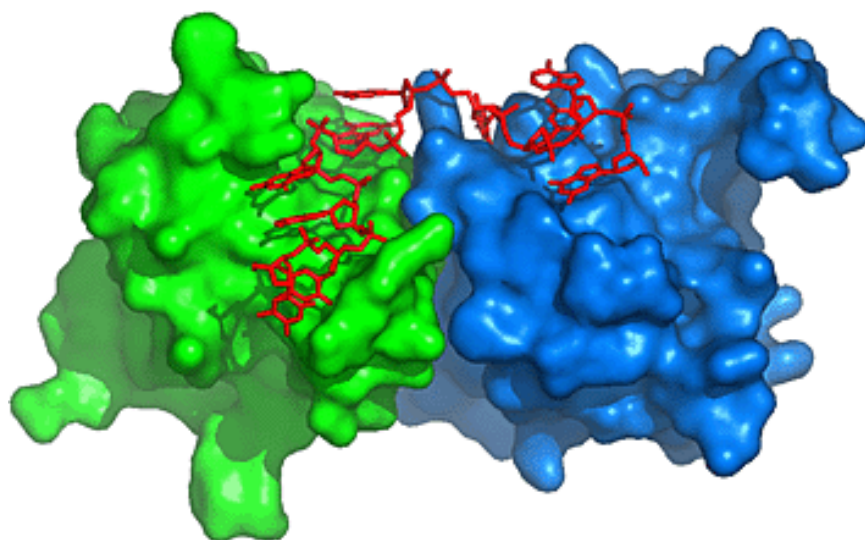


Image Title: The POT1 protein binds to the end of a human chromosome by way of two oligonucleotide/oligosaccharide-binding folds, shown here in green and blue. Single-stranded telomeric DNA is represented in red. - Thomas R. Cech

Scientists have glimpsed the three-dimensional structure of a protein that protects the ends of human chromosomes, a function that is essential for normal cell division and survival. By visualizing the protein as it surrounds the end of a chromosome, the scientists have learned how the protein homes in on a specific DNA sequence and acts like a protective cap to prevent erosion of chromosome ends.

The researchers, led by Howard Hughes Medical Institute President Thomas R. Cech, whose laboratory is at the University of Colorado at Boulder, published their findings in an advance online publication in *Nature Structural and Molecular Biology* on November 21, 2004. Ming Lei and Elaine R. Podell in Cech's lab were co-authors. According to Cech, the findings raise new questions about essential cellular functions taking place at the end of the chromosome.

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— **Thomas R. Cech**

During normal DNA replication, the very ends of a DNA molecule are lost. In order to prevent erosion, chromosomes are capped with a specialized region of DNA known as a telomere — a short, repetitious DNA sequence that does not code for any protein. In humans, an entire telomere is thousands of base pairs long, and is made up of a repeating sequence of six nucleotides. The final 100 to 300 nucleotides at the very end extend beyond the double helix as a single-stranded DNA “tail.” The telomeres of normal cells gradually become shorter and shorter with each cell division, a characteristic sign of cellular aging.

But cells also possess a unique enzyme known as telomerase that can lengthen telomeres by adding DNA to the ends of the chromosome using its own RNA template. In most cells, telomerase activity is very low after embryonic development, and regulation of telomerase is critical, because too much telomerase activity can promote tumor development.

In 2001, Dr. Peter Baumann in Cech's laboratory discovered POT1 (for “protection of telomeres”), the only protein known to bind to human telomeric DNA tails. POT1 plays an important role in capping the ends of chromosomes and in regulating telomere length. “Before that discovery,” he said, “people weren't even in agreement that there was a protein at the very ends of human chromosomes.” At the same time, Cech's team found a version of the POT1 protein in fission yeast. Other versions of POT1 have since been found in plants and mice — each recognizing a telomeric sequence that is unique to that organism.

POT1 is critical to normal cell division and survival; experiments in fission yeast have shown that without it, most cells die immediately. Cells that do manage to survive quickly lose their telomeres, which interferes with normal cell division and eventually leads to massive DNA errors and abnormal, circular chromosomes. In human cells grown in the laboratory, too much POT1 can be disruptive, causing abnormal lengthening or shortening of telomeres.

Prior to determining the structure of human POT1, the researchers' prediction of what it might look like was based on their understanding of the yeast version of the protein. In yeast, POT1 wraps around the end of a chromosome via a region known as an oligonucleotide/oligosaccharide-binding fold (OB-fold) — a shape found in many proteins that recognize and bind to DNA or RNA. The repeating six-nucleotide telomeric unit fits precisely within this fold, with many POT1 molecules binding to each chromosome end.

Cech and his colleagues expected human POT1 to have a similar design, but the results of their biochemical analyses of the protein did not fit easily with this model. For example, when the scientists added the protein to short pieces of DNA containing the six nucleotides that make up a human telomeric repeat, the human POT1 protein bound poorly.

To their surprise, they found that POT1 required a stretch of telomeric DNA containing at least ten nucleotides for efficient recognition and binding of DNA. “We were confused about how ten nucleotides was even a binding site, because it wasn't a multiple of six,” Cech said. “If you need to coat something that has a repeating motif of six, you need to bind some multiple of six.”

To understand how human POT1 recognized and bound to the telomere, the researchers crystallized a form of POT1 bound to the critical ten-nucleotide segment of DNA. They then used x-ray diffraction to reveal the structure of the complex. Unexpectedly, they found that unlike the yeast version of the protein, human POT1 contained two distinct OB-folds. The grooves of the two folds align with one another, forming a continuous channel where the telomeric DNA can fit.

They also learned that while the protein would bind to a ten-nucleotide sequence, the structure could also accommodate twelve nucleotides. “So it turns out it doesn't bind one six, it binds two times six,” Cech said. On a single chromosome end, he said, there might be eight to 24 POT1 molecules coating the DNA tail.

The structure of the complex suggests that the end of the chromosome is tightly protected by POT1, and the researchers were able to verify this with additional biochemical experiments. When the POT1-DNA complex was treated with a solution that would normally modify the DNA at specific sites, no such changes occurred — indicating that those sites were completely enclosed by the POT1 protein.

According to Cech, the findings raise important questions about the regulation of telomerase. When telomeric DNA is buried within POT1, telomerase cannot access the DNA to elongate the telomere. “This is something that could keep the cell from making telomeres all day long,” he said. “We think this is one level at which telomerase is regulated.” Therefore, he said, an important next step will be to determine the cellular mechanism that switches the telomere to the on state so that elongation can occur.

“This is the end of the human genome. If you march out to the ends of human chromosome, what's there? Now we know what is there — at least part of the time,” Cech said. “There may be other states of the telomere, as well, but we think this is right where the action is.”