

Stopping a Force of Nature

Keeping a chromosomal enzyme from its appointed rounds may prevent cancer cells' immortality.

TELOMERES, THE LONG CHAINS OF DNA LETTERS CAPPING THE ENDS OF chromosomes, seem like a Dr. Seuss creation. What they spell out, in the language of DNA, amounts to gibberish. The exact spelling varies from organism to organism but usually consists of repetitions of a single 6- to 10-letter “word.” In human telomeres, the sequence is TTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG... stretching for thousands of letters. ¶ For all their senseless monotony, telomeres play essential roles in cells and organisms. Without them, the genetic legacy of higher organisms could not endure as it does through countless generations. But that same life sustenance that telomeres breathe into species can also bring

death to individual organisms, explains HHMI president Thomas R. Cech, whose laboratory at the University of Colorado studies telomerase, the enzyme that maintains telomeres on chromosomes. “Telomerase is involved in about 90 percent of cancers,” Cech says.

All human cells initially contain telomerase, he explains, but most adult body cells, which typically divide only a limited number of times, lack the enzyme. As those cells age, their telomeres gradually shorten, prompting them to stop growing and senesce. But telomerase becomes reactivated in cancer cells, continually lengthening the telomeres, endowing the cells with immortality and energy-sapping dominion over the body.

Cech’s research team recently made a key finding about a crucial piece of the enzyme, called telomerase reverse transcriptase (TERT). Understanding TERT’s three-dimensional structure could help scientists develop drugs to turn the renegade enzyme off in cancer cells. But until very recently, attempts to grow crystals of the enzyme, required for solving its structure, had failed. “Lots of labs have been trying to crystallize it since we discovered TERT 9 years ago, but

the protein has never been very soluble,” Cech says. When produced in bacteria, the enzyme molecules tend to aggregate into misshapen clumps, called inclusion bodies, which are useless for structural studies.

Finally, Steven A. Jacobs, a Damon Runyon Cancer Research Foundation post-doctoral fellow working in Cech’s lab, took a new tack. His novel method and important findings were reported in February in *Nature Structural & Molecular Biology*. Rather than trying to force the entire protein to crystallize, Jacobs used genetic engineering methods to create more than 10,000 different random

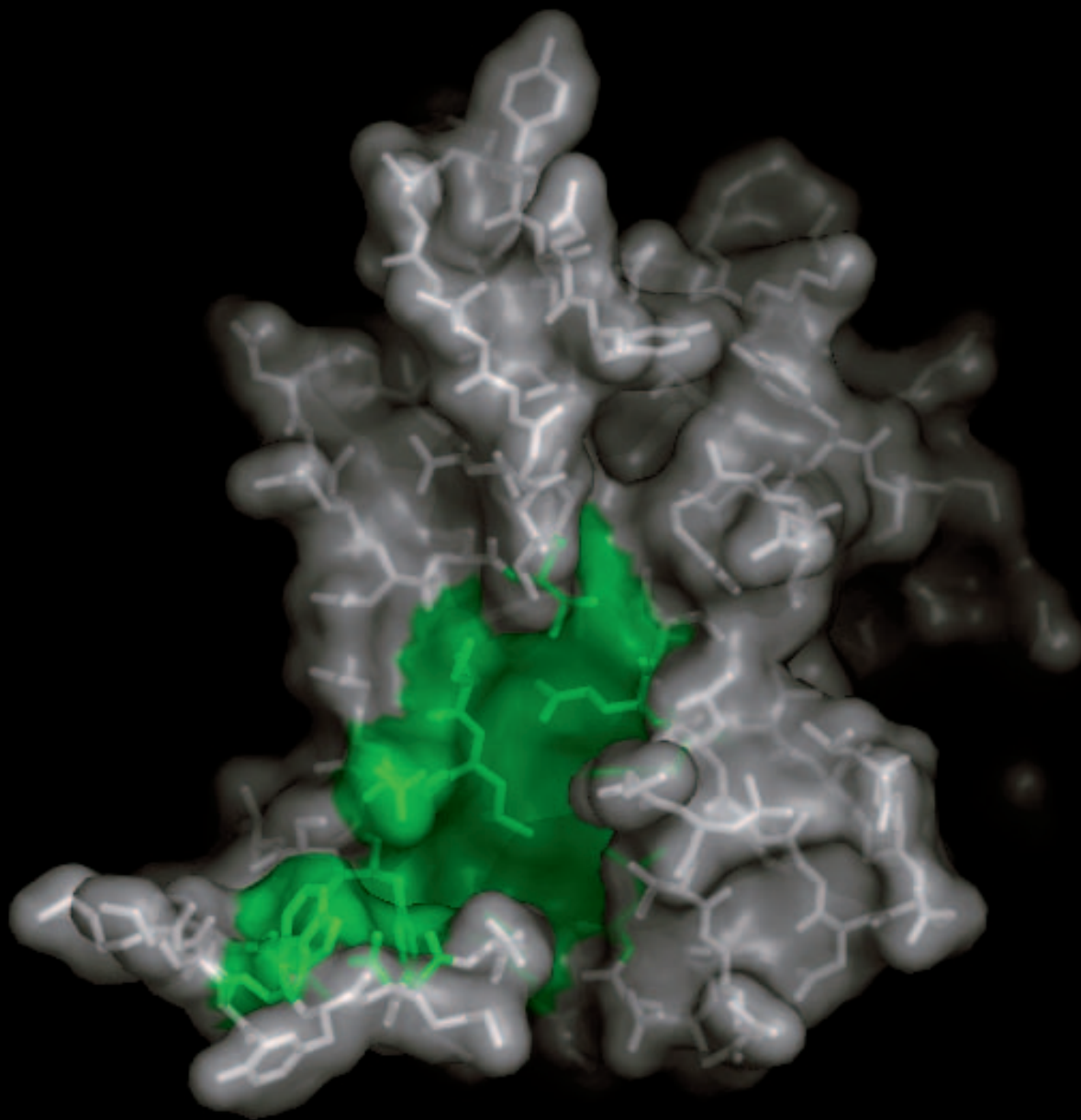
TERT protein fragments, each produced by a different population of *Escherichia coli* bacteria. He genetically fused each fragment to green fluorescent protein (GFP), which glows when illuminated with ultraviolet (UV) light. The GFP would serve as a biochemical beacon, alerting the scientists of any fragments that happened to fold correctly.

As expected, practically all the random fragments misfolded and formed insoluble inclusion bodies. “The inclusion bodies pulled the GFP inside them so that the proteins couldn’t fluoresce nearly as brightly as they could if they were soluble—the GFP essentially goes down with the ship,” Cech explains. But some of the protein fragments formed structures that could fold into their correct shape as they do in the full-length protein, and they did remain soluble within the *E. coli* cells that produced them. “You just have to shine a UV lamp on the Petri dish

THE BIG PICTURE

A Natural End

BECAUSE OF A QUIRK in the way chromosomes replicate, the DNA polymerase enzyme can’t copy the letters at the chromosome tips. Consequently, newly copied chromosomes lose about 50 letters from their ends at every round of replication. >> **TELOMERES CIRCUMVENT** the shortcoming by providing meaningless DNA sequences at chromosome ends. The telomeres sacrifice their own tips to shield the chromosome’s genes from being lost. Over generations, the telomeres of most of the body’s cells steadily shorten, placing the genes in jeopardy of being lost. >> **FACING SUCH LOOMING DISASTER**, most healthy cells stop dividing when their telomeres become too short. But the special cells that go on to create new life, in the form of eggs and sperm, cannot afford to lose genes or to stop dividing. Either would bring the species to extinction. As a solution, cells that produce sperm and eggs continually extend their telomeres with telomerase, ensuring that species can continue into perpetuity.



In this model of part of the telomerase enzyme, green highlights the groove that “anchors” the protein near the chromosome’s tip.

and literally pick the bright green colonies off the plate with a toothpick,” he explains.

Jacobs purified the TERT-GFP fragments from several brightly glowing *E. coli* colonies, and sure enough, one of them crystallized readily, allowing the precise atomic structure of the protein to be determined by x-ray diffraction.

That fragment was from a region of the TERT protein chain called the N terminus. By comparing the sequences of the same region of TERT from several different species, identifying the common amino acid positions shared among all the species, and mapping those positions to their structural model, the researchers inferred that a grooved cleft running through the protein must be important to the enzyme’s function. After changing several of those key amino acids in the full-

length TERT protein, they found that the enzyme lost its ability to assemble telomeres. “So we knew that this domain was necessary for function,” Cech says. They dubbed the fragment the “TEN domain,” for telomerase essential N terminus.

Further experiments showed that the TEN domain clamps onto the DNA near the end of the chromosome, positioning telomerase to begin building telomeres. “It’s a part of the protein that is miles away from the active site [the part that catalyzes the chemical reaction],” Cech says, yet it’s completely essential for activity.” The hope, he says, is that scientists can now develop drugs targeting the TEN domain, preventing it from clamping to the chromosomes in cancer cells, and thereby nixing their immortality. ■

—PAUL MUHLRAD

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TOM CECH