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Groovy Protein Essential for Promoting Cancer Development

Scientists have determined the detailed structure of an essential piece of the telomerase enzyme, an important contributor to the vast majority of human cancers. Understanding the physical shape of the protein has led to a better understanding of how it acts to immortalize cells - and should help scientists design broadly effective cancer drugs.

Until now, the lack of detailed structural information about the enzyme has hindered progress in developing agents to inhibit it, say the researchers, who published their findings in an advance online publication in *Nature Structural & Molecular Biology* on February 5, 2006. Howard Hughes Medical Institute President Thomas R. Cech, whose laboratory is at the University of Colorado at Boulder, led the study, conducted with colleagues Steven A. Jacobs and Elaine R. Podell.

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Cancer researchers have long sought a way to subdue telomerase, an enzyme whose excessive activity contributes to the unchecked growth of as many as 90 percent of human tumors. The enzyme is vital for some rapidly dividing cells - such as those in a developing embryo - where it extends telomeres, the regions of highly repetitive DNA found at the ends of chromosomes. In most healthy adult cells, telomerase is shut off, and telomeres slowly shrink during cell division - a normal process that helps limit cells' lifespan. Cancer cells, however, usually find a way to turn telomerase back on, achieving a dangerous immortality. "Getting telomeres replicated again is required for carcinogenesis to proceed," Cech explained. "It's an essential step in the

development of cancer, and that makes it of a lot of interest therapeutically, because it is a target that could impact a wide variety of cancers.”

Telomerase inhibitors have been in clinical development for many years, but, Cech said, progress has been slow. “The development of anti-telomerase chemotherapeutics has been challenged by the fact that there was no structural basis for thinking about the problem,” he said. “There was no picture in any detail of what any part of this protein looks like.”

Many labs have been working toward developing that picture, but the task has proven challenging. That's because the enzyme tends to clump together once outside of cells, preventing it from forming the ordered crystals necessary for structural studies. Scientists from Cech's lab and others had tried to simplify matters by crystallizing a portion of the protein, but the segments that they selected clumped together just as stubbornly as the whole enzyme.

Jacobs, a Damon Runyon Cancer Research Foundation fellow in Cech's lab and the first author of the paper, developed a new approach. With the help of bacteria and a protein that emits green fluorescent light, Jacobs randomly screened tens of thousands of fragments of the enzyme for one that would lend itself to successful structural analysis. His strategy took advantage of the fact that when multiple copies of the fluorescent protein clump together, the fluorescence is quenched, or extinguished. So Jacobs engineered bacteria to produce fragments of the telomerase enzyme fused to the fluorescent protein. Since telomerase fragments that clustered together would drag along - and quench - their associated fluorescent protein, Jacobs knew that any bright green bacterial colonies were producing protein fragments that remained free. Those rare colonies would be the best candidates for further analysis.

Jacobs performed these experiments on fragments of the telomerase enzyme from a variety of organisms, and found that only a fragment from *Tetrahymena* -- the single-celled organism in which telomerase was first discovered -- would work. The researchers named the protein fragment “telomerase essential N-terminal” (TEN) domain, in reference to its position within the complete enzyme. It took a few more biochemical tricks, but eventually Jacobs crystallized the protein fragment and analyzed it using x-ray diffraction.

Finally, the researchers were able to obtain an extremely detailed three-dimensional map, elucidating the position of each individual atom within the TEN domain. Their studies revealed that TEN was characterized by a deep groove on its surface. “But,” Cech said, “a protein crystal structure

without its relevant partners is not very informative.” So the team went on to do further analysis.

Telomerase has to grab on to the end of a chromosome in order to extend it, and Cech said scientists had previously decided that the region of the protein his lab was now studying might contain the essential “anchor site.” Indeed, the TEN domain was able to grip telomeric DNA in a test tube, and when the researchers made a series of single amino acids changes within the domain, they found that three of these severely affected the binding of the chromosome end. “They turned out to be lined up right within that groove,” Cech said.

The scientists found that these same mutations abolished telomerase's ability to extend telomeres, demonstrating that the groove was important for active telomerase.

“We now have a detailed picture of the part of telomerase that forms this anchor site, and in fact have identified a groove within the protein that is what is really holding on to the end of the chromosome,” Cech said. The very tip of the chromosome must remain free to allow access to the site on the enzyme that directly extends the telomeres, Cech pointed out, so the anchor site secures the DNA molecule nearby, slightly closer to its center.

Cech is optimistic that the new portrait of the TEN domain will speed the development of telomerase inhibitors as chemotherapeutic agents. “A molecule that would sit in that groove - even though it's far away from the active site - looks like it would completely abolish the ability of telomerase to work.” This expands the possibilities for drug design, he said. “Instead of an active site inhibitor, you could screen for a TEN domain inhibitor.”