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Watching a Biological Jigsaw Puzzle Come Together

Scientists have recorded the action involved in assembling telomerase, an enzyme used by cells to protect their genes during the potentially dangerous process of DNA replication. Using a sophisticated technique for tracking structural changes in individual molecules in real time, they have revealed how three of the protein and RNA components of the enzyme come together, altering their shapes along the way to ensure that the next piece will fit.

In humans, telomerase is vital to cells that divide rapidly, such as those in a developing embryo, but is turned off in most healthy adult cells. When the normal controls on telomerase are lost, however, the enzyme can promote cancer by encouraging cells to grow without limits. One of the ways cells are thought to ensure that telomerase activity is kept in check is by controlling how much of the enzyme is assembled, so it is important to understand how that process occurs, the researchers said.

"It's really like a jigsaw puzzle. When you put in one piece, it helps another piece to find its proper place so everything fits."

— Xiaowei Zhuang

For the current study, which was published online on February 25, 2007, in the journal *Nature*, Howard Hughes Medical Institute investigator Xiaowei Zhuang and colleagues tracked the assembly of the version of telomerase found in a single-celled pond organism called *Tetrahymena thermophila*. Michael Stone, a postdoctoral fellow in Zhuang's lab at Harvard University, led the study. Zhuang said telomerase from *Tetrahymena* was a logical place to begin, because its components are structurally simpler and more amenable to single molecule study than the telomerases of higher organisms.

Decades of work from labs around the world has helped explain how telomerase extends telomeres, the regions of highly repetitive DNA found at the ends of chromosomes. Without the enzyme, telomeres slowly shrink during cell division — a normal process that helps limit cells' lifespan. Telomerase relies on two essential components to carry out its work: a protein enzyme called telomerase reverse transcriptase (TERT) and a short

stretch of RNA that serves as the enzyme's instruction sheet.

It only needs these two things for activity, Zhuang explained. However, if you just put the two things together, they will not assemble into the functional structure. There are other helper proteins that are needed. Work in the lab of Kathleen Collins at the University of California, Berkeley, who collaborated with Zhuang's lab on the new research, had found that in *Tetrahymena*, a third molecule — a protein called p65 — promotes telomerase assembly.

We wanted to see how these three things come together by monitoring the assembly of a single complex in real time, Zhuang said. Since p65 does not contribute to telomerase function directly, the scientists suspected it might help choreograph the movements that bring together TERT and the telomerase RNA. Our hypothesis, which seemed quite reasonable, was that p65 binding to the RNA brings two distant sites of the RNA together, and that these two sites will be where the telomerase reverse transcriptase binds, Zhuang said. Without p65, the TERT protein would have a hard time reaching both of those sites. But if you've already brought them together with p65, then TERT can latch on in a much more stable way.

To test their hypothesis, the scientists turned to a technique known as fluorescence resonance energy transfer, or FRET—a powerful method of tracking structural changes in a molecule, pioneered by Stanford scientist Lubert Stryer. Researchers prepare a molecule for FRET analysis by labeling it at precise locations with two fluorescent dyes. Each of these dyes emits a distinct color of light, and one, considered the FRET donor, is able to transfer energy to the other (the FRET acceptor). How much energy is transferred depends on how close together the two dyes are. By measuring how much light each FRET label emits, researchers can directly observe relative changes in distance between the two points on the molecule they are tracking.

Zhuang and her colleagues attached FRET labels near two sites on telomerase RNA that, although distant from one another when the RNA is alone, both interact with an RNA binding site on TERT. When they measured the energy transfer between the two labels, they saw that when the RNA is allowed to interact with p65, these sites become closer together. After the researchers added the TERT protein to the RNA-p65 complex, the shape of the RNA shifted again — reflected by another increase in energy transfer between the dyes.

Once they had determined the FRET level that accompanied each conformation of the RNA, the scientists used that information to track what happened when they combined all three telomerase components. In a soup, we mixed all three together and watched how the FRET changed, Zhuang explained. It was really quite remarkable - we could directly observe how each individual complex comes into shape and see that the p65 binds first, and then causes an intermediate conformation of the RNA where the two TERT binding sites are indeed brought closer together. And then the TERT binds and snatches the RNA into its final functional form. So you can see this very nice stepwise assembly process in real time. It's really like a jigsaw

puzzle, she said. When you put in one piece, it helps another piece to find its proper place so everything fits.

Zhuang noted that with the FRET technique, the researchers not only learned the individual steps of telomerase assembly, but were also able to glean specific structural information. By moving the dyes around on the molecule, we found out which part of the conformational change is essential for this assembly process, she said. She explained that p65 causes the RNA to become compact in a precise location. Scientists had long known that the RNA sequence at this point - comprised of just two nucleotides - had remained remarkably unchanged throughout evolution, but it had been unclear why. The new results suggest that the nucleotides are needed to stabilize the RNA's critical compacted structure, ultimately enabling TERT binding.

Stone noted that *Tetrahymena* telomerase has strong similarities to human telomerase, and it may be reasonable to expect that the two share key features of their assembly processes. It's widely accepted that there are proteins involved in the assembly of ribonucleoproteins - not just telomerase — that play a role that is likely to be very similar to that of p65. That is, they help macromolecular structures coassemble into active particles, he said. That's not an easy task — there are a lot of things that can go wrong. So having these accessory proteins can ensure that an enzyme is proceeding along an appropriate path.

Zhuang said the next step is to extend these studies to telomerase in more complex organisms. If you study a molecule that's of such medical importance, she said, eventually you have to link it to *Homo sapiens*, not just to *Tetrahymena*. In these more complicated systems, it's much harder to guess what is going on in the assembly process. But by directly watching things as they happen, this sort of powerful approach will give a lot of new insights.