For DNA Repair Machine, it's all About Flexibility

Lawrence Berkeley National Laboratory

In a discovery that could lead to new ways to fight cancer and other diseases such as cystic fibrosis, scientists from the U.S. Department of Energys Lawrence Berkeley National Laboratory (Berkeley Lab) and the Scripps Research Institute determined that a cells speedy ability to repair damaged DNA relies on the remarkable flexibility of a molecular motor.

Using the Advanced Light Source, a synchrotron located at Berkeley Lab that generates intense x-rays to probe the fundamental properties of substances, the researchers determined the precise location where two components of a DNA repair machine called MRN attach to one another. To their surprise, they also found that one of these pieces, a molecular motor called Rad50, is as flexible as a snake before DNA repair begins, and then clamps shut like a pair of pliers once it binds with energy-giving ATP and initiates repair.

"We had no idea this motor is tethered to the repair machine in such a flexible way," says John Tainer of Berkeley Labs Life Sciences Division and the Scripps Research Institute in La Jolla, CA. Tainer co-led the research with Paul Russell of the Scripps Research Institute. Their research is published March 27 in an advance online edition of the journal *Nature Structural and Molecular Biology.*

The molecular motors never-before-seen ability to twist and turn helps explain how MRN (also known as Mre11-Rad50-Nbs1) stays at the ready for almost any type of DNA repair job that comes its way, no matter how complex. This insight will help scientists better understand how the repair mechanism fends off cancer in healthy people, and conversely, how it helps cancer cells resist chemotherapy. The latter could enable scientists to develop more effective cancer therapies with fewer side effects.

The discovery also sheds light on how a superfamily of molecular motors called ABC ATPase, of which Rad50 is a member, is versatile enough to drive many biological processes in addition to DNA repair. The family of motors is found in cystic fibrosis transmembrane receptors, as well as in cellular efflux pumps that enable some disease-causing organisms to resist a wide swath of drugs.

"This superfamily motor is so versatile because its flexibly tethered," says Tainer. "Before this research, we believed it only went from open to closed. But now we know it can be open, closed, and anywhere in between."

The research is the latest advance by Tainer and colleagues to understand how MRN rushes in to repair damaged DNA in cells. The first-responder machine zeroes in on the gravest kind of breaks in which both strands of a DNA double helix are cut. It then stops the cell from dividing and launches one of three repair pathways, including the error-free DNA repair process called homologous recombination, which

replaces defective genes. If unrepaired, double strand breaks can lead to the proliferation of cancer cells.

Their tool of choice in this endeavor is an Advanced Light Source beamline called SIBYLS, which can capture incredibly high-resolution images of crystallized proteins, down to individual atoms. The beamline is also equipped with small angle x-ray scattering. This technique can image a protein in its natural state, such as in a solution, and at a spatial resolution of about 10 angstroms, which is small enough to determine a proteins three-dimensional shape.

"The beauty of this dual beamline is that we can merge high-resolution crystallography details with the solution conformations of small angle x-ray scattering," says Gareth Williams, a scientist with Berkeley Labs Life Sciences Division who participated in the research.

The scientists used SIBYLS to solve four new structures of Rad50 motor bound to the Mre11 protein at key stages, such as before and after DNA repair. They studied proteins from a single-celled microorganism called *Pyrococcus furiosus***.** These four structures allowed the researchers to pinpoint, for the first time, exactly where Rad50 connects to Mre11. It also revealed a Rad50 motor that is anything but static.

"This work changes our understanding of ABC ATPases," says Tainer. "Its like we are going from our understanding of an elephant by observing it in the natural history museum, with its trunk frozen in the air, to watching it in the zoo. Now we realize the trunk moves and pulls out trees and such."

The scientists also mutated the residues that connect Rad50 to Mre11 in yeast cells. They found that these cells became very deficient in repairing DNA double-strand breaks.

"This tells us that the region where Rad50 binds to Mre11 is conserved in more complex forms of life such as humans," says Williams. "And this hints at ways to improve cancer therapy by hobbling the structures ability to come together."

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