First look at an enzyme target for antibacterial and cancer drugs

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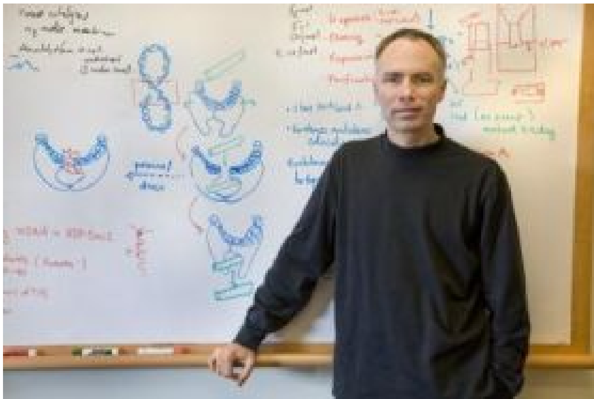

James Berger, with Berkeley Lab's Physical Biosciences Division, produced the first 3-D structural images of a DNA-bound topo II enzyme that's responsible for untangling coiled strands of chromosomes during cell division. Credit: Photo by Roy Kaltschmidt, Berkeley Lab

The veil has finally been lifted on an enzyme that is critical to the process of DNA transcription and replication, and is a prime target of antibacterial and anticancer drugs. Researchers with the U.S. Department of Energy's Lawrence Berkeley National Laboratory and the University of California at Berkeley have produced the first three-dimensional structural images of a DNA-bound Type II topoisomerase (topo II) that is responsible for untangling coiled strands of the chromosome during cell division.

Preventing topo II from disentangling a cell's DNA is fatal to the cell, which is why drugs that target topo II serve as agents against bacterial infections and some forms of cancer. This first ever structural image of topo II should help in the development of future antibacterial and anticancer drugs that are even more effective and carry fewer potential side effects.

"Topo II has been called nature's magician because it literally can move one DNA segment through another," said James Berger, a biochemist and structural biologist who led this research. "The enzyme cleaves a double-stranded DNA, passes a second duplex through the break, and then immediately repairs the broken strands. This enables topo II to control the topology of DNA for chromosome segregation and disentanglement."

Berger holds a joint appointment with Berkeley Lab's Physical Biosciences Division, and UC Berkeley's Department of Molecular and Cell Biology. He and Ken Dong, a graduate student in his research group, co-authored a paper reporting this research in the December 20, 2007 issue of the journal *Nature*. The paper is titled: "Structural basis for Gate-DNA recognition and bending by type IIA topoisomerases."

Using the exceptionally bright and intense beams of x-rays generated at Berkeley Lab's Advanced Light Source (ALS), Berger and Dong obtained high resolution, 3-D crystallography images of the DNA binding and cleavage core of a topo II enzyme taken from yeast as it interacted with a segment of DNA. The images revealed that topo II causes a sharp bend - 150 degrees or more - in the DNA segment at the point where it is cleaved. The near folding-in-half of the DNA segment helps enable topo II to recognize where it should disentangle DNA strands.

"Large conformational changes in the topo II accompany the DNA

deformation, creating a bipartite catalytic site that positions the DNA backbone near a reactive tyrosine and coordinated magnesium ion," said Berger. "Remarkably, this configuration turns out to also closely resemble the catalytic site of certain type I topoisomerases, which reinforces the evolutionary link between what are otherwise structurally and functionally distinct enzymes."

If the DNA in a single set of human chromosomes is stretched out and joined together, it measures about two meters in length. In order to be packed within the tiny confines of a cell's nucleus, all of this double-stranded DNA must be tightly bundled by a process known as supercoiling. During mitosis, the process by which a dividing cell duplicates its chromosomes and distributes them equally between two daughter cells, these coils of DNA give rise to knots and jumbles that must be unlinked. Failure to properly do so can give rise to chromosome breaks, which in turn can lead to genome instabilities and cell death.

"You can think of DNA in the cell as the ultimate extension cord or set of holiday lights which, no matter how carefully you organize them, inevitably must be unraveled before they can be used," said Berger. "Topo II performs this unraveling for DNA in the cell."

Despite the important role and extensive study of topo II, structural information and how the enzyme is able to perform its magic have remained a mystery. The problem was that until the advent of synchrotron light sources such as the ALS, researchers lacked the tools to perform sufficiently high-resolution crystallography. The research of Berger and Dong was performed at ALS Beamline 8.3.1, which is powered by a superconducting bend magnet or "superbend," and which has experimental facilities that offer both multiple-wavelength anomalous diffraction (MAD) and monochromatic protein crystallography capabilities.

“The high brightness of the x-ray beams and the experimental capabilities at Beamline 8.3.1 were critical to our success,” said Berger. “Another big plus was the sheer user friendliness of the ALS, which greatly facilitates data collection and imaging.”

Based on the structural images he and Dong created, Berger believes that topo II employs a “two-gate” mechanism to carry out its tasks. The upper domain of topo II opens to admit a segment of DNA and transport it to the enzyme’s core where the segment is folded. A second DNA segment is then admitted and the upper domain gate closes. This closing of the upper gate triggers the cleavage of the bent DNA segment and the subsequent transport of the second DNA segment through the break. When the gate in topo II’s lower domain swings open, the second DNA segment is released and the cleaved DNA segment is reconnected.

“In many ways, the enzyme works like a set of canal locks, opening and closing and certain protein interfaces, or gates, to control the passage of one DNA segment through another without accidentally letting go of the DNA and breaking the chromosome irreversibly,” Berger said. “Our structural studies should serve as a useful platform for future efforts to understand the chemical basis of DNA cleavage, and for efforts to understand and improve anti-topoisomerase therapeutics.”

Antibacterial and anticancer drugs that target topo IIs and other topoisomerases, such as the quinolone family of antibiotics (of which the commonly-used ciprofloxacin is a member), work by preventing the enzymes from completing their tasks. In the case of topo IIs, the cleaved segments of DNA remain attached to the topo II so that they cannot be resealed. The number of damaged DNA strands mount until the cell is destroyed. Because the targeting of these drugs has not been optimal, there have sometimes been side effects that pose their own health risks, including secondary cancers with anti-topoisomerase chemotherapeutics. There also can be issues with the toxicity of antibiotics that target

bacterial type II topoisomerases, as well as microbial resistance.

"In some respects, it's amazing that the anti-topo II drugs have been so effective," said Berger. "To the credit of the biochemists and chemists, their discovery and refinement of these compounds have already made a remarkable therapeutic impact. Yet, to best of my knowledge, all of the work on these drugs has been done without a good picture of how type II topoisomerases engage DNA. Our new structural knowledge fills that hole, and should be of significant help for overcoming resistance and for guiding the development of future anti-topo II drugs with improved efficacy."

Berger and his research group are now looking into producing crystallographic images of topo II as it interacts with antibacterial and anticancer drugs to determine what the rules for engagement are.

Source: Lawrence Berkeley National Laboratory

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