Preventing cells from getting the kinks out of DNA

21 May 2010, By Robert Sanders

(PhysOrg.com) -- Many standard antibiotics and anti-cancer drugs block the enzymes that snip the kinks and knots out of DNA -DNA tangles are lethal to cells - but the drugs are increasingly encountering resistant bacteria and tumors. A new discovery by University of California, Berkeley, biochemists could pave the way for new research into how to re-design these drugs to make them more effective poisons for cancer cells and harmful bacteria.

"The development of the anti-bacterial and anti-tumor agents that target these enzymes thus far has been done entirely in the absence of any visualization of how these drugs actually interact with the protein itself. And they have done remarkably well," said James Berger, UC Berkeley professor of molecular and cell biology. "But we have increasing problems of resistance to these drugs. Being able to see how these drugs can interact with the enzyme and DNA is going to be critical to developing the next generation of therapeutics that can be used to overcome these resistance problems."

Berger and colleagues at Emerald BioStructues of Bainbridge Island, Wash., and Vanderbilt University in Nashville, Tenn., report their new findings in a paper to be printed in the journal *Nature* and made available this week as an advance online publication.

The tangles in DNA, like those in a string of holiday lights, are a result of packing some six feet of DNA into a cell nucleus so small that it is invisible to the naked eye. Every time a cell divides, it has to unpack, duplicate and repack its DNA, generating about a million tangles among the newly-copied chromosomes in the process.

As Berger has shown in previous work, enzymes called topoisomerases home in on the sharp turns in a knot and then progressively snip the DNA, unloop it, and restitch it flawlessly. If, however, the enzyme slips up, that one snip can turn into a potentially mutagenic or cell-killing DNA break.

While the protein structure of these topoisomerases is known, the details of the chemical reactions that take place between the enzyme and DNA, and their reaction with the drugs that bind both, remain a mystery, Berger said. In fact, one of the main puzzles is why antibiotics like ciprofloxacin (Cipro) and anticancer drugs like etoposide, which vary widely in structure, have the same effect: jamming the enzyme and causing a break in the double-stranded DNA helix.

Berger and his colleagues found a way to obtain a picture that shows the interaction of the protein bound to DNA. The next step is to do the same for a drug bound to the protein/DNA complex, getting an image of exactly how these drugs interfere with the knot elimination machinery.

"The technique we used to trap this complex so that we could actually crystallize it and image it we think now gives us a handle on how to go after drug-bound complexes of human topoisomerases that have long eluded the field," said Berger, who also is a staff scientist at Lawrence Berkeley National Laboratory (LBNL).

The scientists' new picture of the enzyme bound to DNA also turned up something totally unexpected. Most enzymes that bind DNA to snip or stitch it together use two metal ions - typically two magnesium ions - to catalyze the reaction. Berger found that type II topoisomerases, which target double-stranded DNA, make use of only one of their two magnesium ions and instead use the amino acid arginine as their second catalytic center. The second magnesium merely provides structural integrity to the protein.

"We stumbled upon a new kind of cleavage mechanism for DNA, an example of a protein that uses a completely new approach for the same

mechanism," Berger said. "It speaks to the evolutionary plasticity and adaptability of nature that continuously amazes us with finding new ways to carry out reactions that it needs to perform."

Berger now plans to use his trick to trap the enzyme on a short segment of DNA, allowing him to collect enough to crystallize and analyze in an X-ray beam from LBNL's Advanced Light Source, to trap both drug and enzyme on DNA. Once crystallized and imaged, he will have the first full picture of a topoisomerase interacting the way it does in a real cancer cell or microbe.

Berger's coauthors are UC Berkeley graduate student Bryan H. Schmidt; chemist Alex B. Burgin of Emerald BioStructures; and biochemists Joseph E. Deweese and Neil Osheroff of Vanderbilt University School of Medicine. The X-ray crystallography of the protein/DNA complex was conducted in Stanley Hall at the UC Berkeley branch of the California Institute for Quantitative Biosciences (QB3), with which Berger is affiliated.

Provided by University of California - Berkeley