DNA repair changes with the flip of a switch

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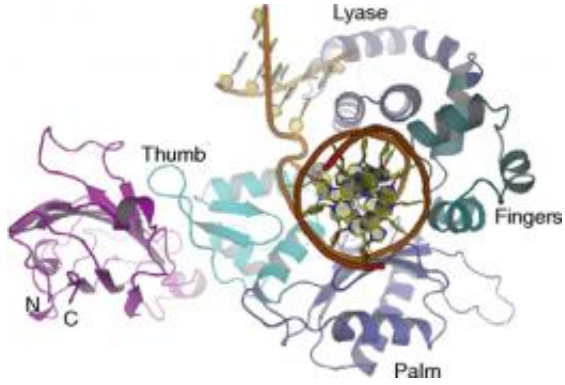

Model of the complex formed by XRCC1's N-terminal domain (in purple), damaged DNA (in orange), and DNA polymerase ß (with component parts thumb, lyase, fingers, and palm labeled).

(PhysOrg.com) -- The DNA blueprint in each human cell undergoes about 100,000 damaging events every day. Because a cell's survival depends on the repair of these damaged molecules, each injury signals a team of proteins to work together to fix the mutated DNA.

"DNA repair is a very important process," said Robert London, a researcher from the National Institute of Environmental Health Sciences (NIEHS). "At NIEHS, the reason we're interested in repair is that one of the effects of environmental toxins can be to damage DNA. We want to understand how the cell deals with damage."

In pursuit of a better fundamental understanding of DNA repair, London

and Matthew Cuneo, a member of London's NIEHS team, use the NSLS to image a large, multi-part molecule called a scaffolding protein. The molecule, called XRCC1, orchestrates DNA repair by holding the other repair proteins together in a multi-molecule complex.

"It's just holding onto all these other proteins and onto the damaged DNA," explained London.

A technique called SAXS, for small-angle x-ray scattering, allows the researchers to take a low-resolution picture of the molecule, obtaining a macroscopic image rather than the details of its fine structure. In SAXS, a protein in solution (the state in which it exists in the body) can be targeted with x-rays, which then scatter and hit a detector, allowing researchers to reconstruct an image of the protein's surface.

Using SAXS, London and Cuneo studied a subsection of XRCC1 called the N-terminal domain. The N-terminal domain interacts with DNA polymerase ß, a protein that actively repairs damaged DNA.

Their study, published in the April 13, 2010 edition of the journal Proceedings of the National Academy of Sciences, shows that this interaction can change with the flick of a biological switch.

The N-terminal domain contains a "disulfide switch," a potential bond whose formation changes the molecule's secondary structure. When the bond forms, the switch is flipped on, transforming the molecule from a reduced state to an oxidized state. In the reduced state, one of the amino acids that make up the N-terminal domain has electrons to spare. This makes the amino acid very susceptible to oxidation, a process in which it shares these electrons with another amino acid, forming a disulfide bond between the electron sharer and receiver and transforming the N-terminal domain into its oxidized state.

"We've never observed a protein undergo such a dramatic change in structure," said Cuneo. When oxidized, the entire N-terminal domain's structure shifts, altering the shape of its surface. "When you change the surface drastically, it affects what sticks to it."

In this case, the oxidized surface binds more strongly to DNA polymerase ß than does the surface of the reduced form. But what conditions flip the switch in the first place, and what is the effect of the stronger bond on the other proteins and on the cell's overall health?

"It's possible that what we're seeing here is part of the cell response to oxidative stress," suggested London. Oxidative stress causes DNA damage, so the disulfide switch may turn on in its presence to step the repair process into overdrive.

But this is by no means the only possibility. London added.

"It's also possible that the switch has another significance related to the repair process," he said. "All this suggests strongly that the switch is a physiologically significant event, but we need to figure out what activates it and why."

Discovering the disulfide switch's role would help improve scientists' understanding of how DNA repair works. This knowledge could lead to better treatments for disease.

"DNA repair is relevant to environmental health, to cancer, and to other disease issues such as mutations," London said.

But before scientists begin seeking a cure for cancer, they need to gather more knowledge about the repair process. Next, Cuneo and London will study both forms of the N-terminal domain while its disulfide switch is locked in place, to see how each form interacts with other proteins and

affects the health of a cell.

More information: M.J. Cuneo and R.E. London, "Oxidation State of the XRCC1 N-terminal Domain Regulates DNA Polymerase ß Binding Affinity," PNAS,, 107(15), 6805 (2010).

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