DNA repair proteins monitored at double-strand break

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Investigators at St. Jude Children's Research Hospital had a molecule's eye view of the human cell's DNA repair kit as it assembled on a double-strand break to link together the broken ends. Double-strand breaks are ruptures that cut completely across the twisted, ladder-like structure of DNA, breaking it into two pieces.

Using a technique developed specifically for this project, the St. Jude researchers could determine when repair proteins arrived at or around the DNA break and evaluate its repair—even when particular proteins shifted away from the break to make room for others. A report on this work appears in the May 7 online issue of *Nature Cell Biology*.

The findings are important because disruption of the precise movement of these repair proteins can cause mutations, cell death or cancer, and the ability to track the process so closely will give researchers critical insights into what can go wrong with DNA repair. This could lead to novel ways to make cancer cells more sensitive to therapy by blocking their ability to repair double-stranded breaks caused by chemotherapy or radiation. It could also suggest new strategies for enhancing repair of double-stranded DNA caused by radiation, natural oxidants in food or the body and other toxins that can cause disease and aging.

"Prior to this work, there was no practical and efficient way to find and study the DNA repair proteins that organize themselves on and around a double-strand break in human cells," said Michael Kastan, M.D., Ph.D., St. Jude Cancer Center director. "Our approach solved that problem and

allowed us to document the cell's response to double-strand DNA breaks over time. The technique provides significantly more information about the proteins that repair DNA than is possible using the standard microscope-based approach previously used for such work." Kastan is the paper's senior author.

A deficiency in two of these repair proteins, ATM and NBS1, leads to defects in double-strand break repair by disrupting the signaling processes triggered by the break. "A lack of functioning ATM causes ataxia-teleangiectasia, a disease that causes a variety of debilitating problems, such as neurodegeneration, cancer and sensitivity to irradiation leading to double-strand breaks that are not repaired," Kastan said. "And a lack of NBS1 causes Nijmegen breakage syndrome, another disease that leaves its victims at high risk for cancer and higher sensitivity to DNA-damaging radiation. So this work has important medical implications for these and other diseases linked to disruption of double-strand break repair."

The assay, developed by Elijahu Berkovich, Ph.D., in Kastan's laboratory, demonstrates how key repair proteins, such as ATM, NBS1, XRCC4 and gamma-H2AX, interact to coordinate repair of double-strand breaks. For example, the investigators showed that NBS1 recruits ATM to the break; and that ATM and NBS1 cooperate to disrupt nucleosomes—the compact packages formed when strands of DNA wind around proteins, called histones, like thread around a spool. Disruption of the nucleosome at the site of a double-strand break allows the DNA to unravel and expose the area to repair proteins; the loss of functioning ATM and NBS1 blocks this important process. The team also showed that both NBS1 and ATM are needed to ensure that the repair factor, XRCC4, arrives at the double-strand break to help repair the damage.

In addition, the investigators showed that ATM initially binds to DNA

both at the site of the break as well as on each side of it. However, XRCC4 later takes the place of ATM molecules at the break while the ATM molecules on either side of the break stay in place. The researchers suggested that ATM had been displaced or moved so that the repair proteins could gain access to the damaged DNA site.

The findings also suggested that before ATM can move to the double-strand break, it must first become activated so it can trigger a critical series of signals linked to DNA repair. Inactive ATM exists as a pair of these molecules linked together. Kastan previously reported in the journal Nature how the inactive ATM molecules separate from each other in response to a double-strand break (http://www.stjude.org/media/0,2561,453_5484_3126,00.html).

To control when and where the double-strand breaks occurred during the study, the researchers used an enzyme called I-PpoI, which naturally seeks certain DNA areas to cut. The investigators modified I-PpoI so that they could better control when the enzyme moves into the nucleus and cleaves the DNA. The team then used a biochemical technique called chromatin immunoprecipitation to collect and identify repair proteins and show where each one bound to the DNA.

Source: St. Jude Children's Research Hospital