Molecule necessary for DNA repairs also halts them

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(Phys.org) -- Repairing DNA breaks can save a cell's life—but shutting off the repair machinery can be just as critical. How cells accomplish this feat was unknown. However, new research by Johns Hopkins scientists, published in the February 22 issue of Nature, suggests that shutting down the repair machinery relies heavily on the same molecule used to start repair in the first place.

According to study leader Cynthia Wolberger, Ph.D., a professor in the Department of Biophysics and Biophysical Chemistry at the Johns Hopkins University School of Medicine, both sides of a DNA strand's double helix can break for a number of reasons, including exposure to ultraviolet light, other kinds of radiation, or certain chemicals. These big breaks can be lethal to cells if left unrepaired, or they can cause cancer or other diseases if repairs are shoddily done. Consequently, summoning repair machinery to break sites to quickly and effectively mend these fissures is critical to keeping cells alive and healthy. However, if this repair machinery keeps running after the task is completed, she explains, it can wreak havoc in a cell by constantly trying to mend breaks that are normal parts of cellular housekeeping and necessary for gene expression.

The repair process for double-strand DNA breaks starts when a molecule called ubiquitin—best known for its role in tagging unneeded proteins for the cellular trash bin—forms chains with the amino acid lysine, which attaches to DNA break sites and serves as a flag to signal repair machinery. A protein called UBC13 is necessary to form these chains. Previous research suggested that a deubiquitinating enzyme called

OTUB1 plays an important role in halting chain formation, but unlike other, similar enzymes, it didn't appear to work by cleaving ubiquitin chains or removing ubiquitin from other proteins.

To figure out what's really taking place, Wolberger and her colleagues started with a biochemical analysis of OTUB1. They found that this enzyme contains two different binding sites for ubiquitin: one on a bulky, globular section, and a second on a tail that extends from this globular section. When the researchers used a chemical trick to simulate ubiquitin binding in the globular section, tests showed that this binding was necessary to activate OTUB1 to inhibit chain formation.

"The real inhibitor isn't just OTUB1. It's OTUB1 bound to ubiquitin," Wolberger explains.

Further experiments showed that the second ubiquitin binding site on OTUB1 appears to be meant for ubiquitin carried by UBC13. The researchers found that when OTUB1 forms a complex with UBC13 by attaching to this second ubiquitin, it knocks off a subunit on UBC13 that's critical for it to function.

The researchers' next step was figuring out how this subunit gets physically removed from UBC13. By working out the 3D structure of activated OTUB1, they found that free ubiquitin bound at the globular site triggers shape changes necessary for OTUB1 to bind to UBC13 by affecting the position of the enzyme's tail. But when this tail binds to UBC13, its position bumps into another subunit called UEV1a, pushing it off UBC13 completely.

Without this subunit, UBC13 can no longer form ubiquitin chains—and without these flags for repair machinery, DNA repairs halt in the cell, Wolberger says. OTUB1 also prevents UBC13 from getting recruited to DNA break sites, further interfering with its activity.

Besides solving a cell biology mystery, Wolberger explains, the finding could eventually form the basis for creating drugs that can help cells repair DNA more efficiently after damage. By crafting molecules that bind to OTUB1 or UBC13, researchers might be able to interfere with this normal shut-off process in select situations, keeping DNA-fixing machinery running longer.

The finding also suggests that other enzymes in cells, especially those involved with ubiquitin, might have many roles that remain undiscovered.

“There are over 100 deubiquitinating enzymes in the genome that we know of,” Wolberger says, “but we still don’t know much about what they do.”

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Other Hopkins researchers on this paper include Reuven Wiener, Xiangbin Zhang and Tao Wang.

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