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# A Critical Cog in the DNA Repair Machinery: Understanding RAD51AP1

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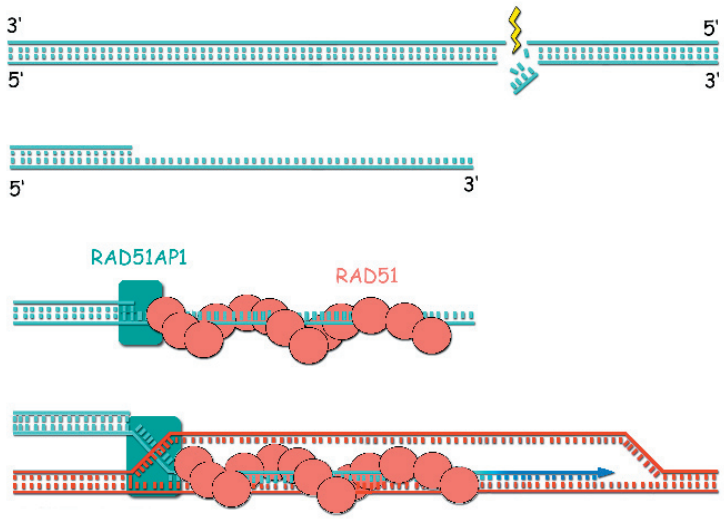
In recent studies of a protein named RAD51AP1, researchers in Berkeley Lab's Life Sciences Division (LSD) and their colleagues at Yale University have discovered how an important cog in the machinery of DNA repair helps promote the vital biological process known as homologous recombination, which is essential for maintaining intact genomes and indispensable for tumor suppression in humans.




*From top: After a double-strand break, the severed DNA is processed (by proteins not shown) to leave dangling ends in the downstream 3' direction. RAD51 forms one of these ends into a presynaptic filament, which seeks out a sequence homologous to (same as) the damaged DNA on the neighboring chromatid. The filament invades the intact strands and opens a D loop, which the broken strands then use as templates to repair their sequence. RAD51AP1 appears to play a role in finding the homologous sequence and opening the D-loop.*

A severe kind of chromosome damage known as a double-strand break, where both backbones of the DNA double helix are snapped—either by external factors like radiation or internal ones like reactive oxygen molecules—occurs about 10 times in every cell division; the cell's need for highly accurate repair is constant. In eukaryotes (organisms whose cells have nuclei), RAD51 is the protein that carries out double-strand-break repair by homologous recombination. But RAD51 can't do the job by itself; a score of other proteins cooperate in the RAD51 complex, and learning how each functions is a field of intense study.

"We became interested in RAD51AP1—the AP stands for an 'associated protein' of RAD51—when we realized that it might have a role like that of BRCA2, an important breast cancer suppressor," says molecular biologist David Schild of LSD, who has long studied RAD51 and its associated proteins, and in whose laboratory much of the recent studies were done. "We didn't know it when we started, but RAD51AP1 is extremely important to this process."

Cell biologist Claudia Wiese, a scientist in the Schild lab, led a Life Sciences Division team in studies investigating the role of RAD51AP1 in cultures of human cancer cells at Berkeley Lab. Meanwhile Eloïse Dray, a postdoctoral fellow in Patrick Sung's lab in the Department of Molecular Biophysics and Biochemistry at Yale University, led in vitro studies. The collaborative aspects of the research were undertaken as part of a larger program in the Structural Cell Biology of DNA Repair Machines, sponsored by the National Cancer Institute and headed by John Tainer and Priscilla Cooper of Berkeley Lab.

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### *Fixing double-strand breaks*

The RAD51AP1 research focused on the steps that initiate double-strand repair by homologous recombination, beginning with the formation of a "presynaptic filament" and the subsequent formation of a "D loop" where the repair proceeds.

To repair a double-strand break through homologous recombination, the break is first processed to leave a dangling end in the downstream or 3' direction of one of the broken strands. Individual RAD51 proteins wind around this long single strand to form a presynaptic filament, which then seeks out and fastens to a corresponding intact stretch of DNA on the broken strands' sister chromosome. The RAD51 complex proceeds to pry up one of the intact sister strands, opening the DNA to form a D-shaped loop, whereupon the broken strands synthesize replacement DNA for themselves by using the exposed, unbroken sister strands as templates.

The research of Schild, Wiese, and their colleagues began by investigating what happens when RAD51AP1 is depleted in a cell. What they discovered, somewhat to their surprise, was how vital RAD51AP1 is in making homologous recombination work effectively.

"We were using human cell lines and couldn't directly knock out the gene for RAD51AP1," Wiese explains. "So to deplete the amount of RAD51AP1 in the cells, we chose several small RNAs that could interfere with the expression of the gene."

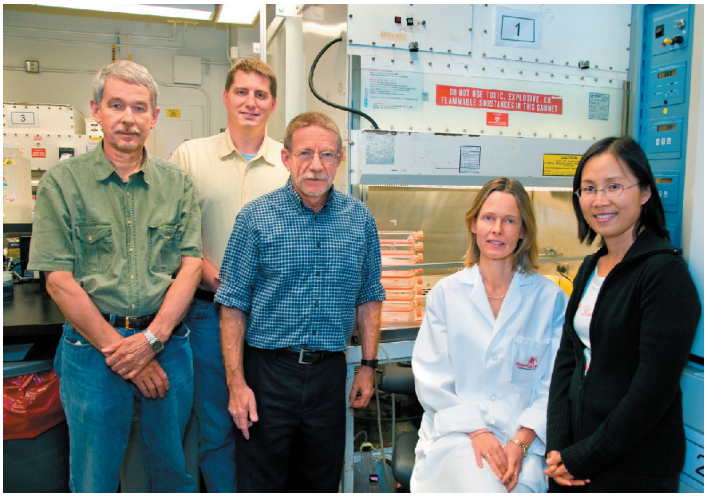
The results were dramatic. Cells of the HeLa line whose RAD51AP1 levels had been depleted by interfering RNAs were more than twice as sensitive to toxins like mytomycin C, which stresses the genome. Breaks in chromatids (the chromosomal arms) increased both spontaneously and in response to DNA-damaging treatment. The cells' efforts to repair these double-strand breaks went awry, resulting in mismatches of the broken ends.

An additional assay was used to confirm RAD51AP1's close association with RAD51 during the repair process, employing cells that incorporated a gene fusion containing the green fluorescent protein (GFP). This green-glowing reporter verified that RAD51AP1 formed part of the RAD51 complex during homologous recombination.

### *Where and when RAD51AP1 goes to work*

Further tests narrowed the scope of RAD51AP1 activity. Whereas studies of other proteins associated with RAD51 had indicated that these participate in processes occurring before the formation of the presynaptic filament, the researchers discovered that RAD51AP1, unlike the others, worked after the formation of the filament.

"Our collaborators found that RAD51AP1 can bind to either single-strand or double-strand DNA, but prefers double-strand DNA," Wiese says. "This suggests that RAD51AP1 may be involved in the homology search—the search for a matching sequence in the undamaged chromatid, which is needed to act as a template for the repair. Our collaborators also found that RAD51AP1 greatly enhances RAD51's ability to form the D-loop"—opening the chromosome and exposing the template sequences.



*Members of David Schild's group who worked on the RAD51AP1 analysis were Bjorn Rydberg, Torsten Groesser, Schild, Claudia Wiese, and Miaw-Sheue Tsai. Not pictured is Gareth Williams.*

*(Photo Roy Kaltschmidt)*

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Homologous recombination is not solely a method of repairing double-strand breaks, but also plays a role in other DNA repairs, such as fixing DNA interstrand crosslinks. Homologous recombination is also vital in meiosis, the double cell division that results in the production of egg or sperm cells.

What the researchers found is that RAD51AP1 works to enhance the activity of RAD51 during homologous recombination, and that it performs this function during the search for matching sequences and the formation of the D-loop.

“Our findings are important in two ways,” says Wiese. “Fundamentally, they help us better understand how homologous recombination can fail and result in mutations that can lead to cancer. Second, by understanding the role of RAD51AP1 in homologous recombination, we may be able to exploit this knowledge for new kinds of cancer therapy.”

***Additional information***

“Promotion of homologous recombination and genomic stability by RAD51AP1 via RAD51 recombinase enhancement,” by Claudia Wiese, Eloïse Dray, Torsten Groesser, Joseph San Filippo, Idina Shi, David W. Collins, Miaw-Sheue Tsai, Gareth Williams, Bjorn Rydberg, Patrick Sung, and David Schild, appears in the 9 November, 2007 issue of *Molecular Cell* and is available online to subscribers at http://dx.doi.org/10.1016/j.molcel.2007.08.027.