DNA repair machine on the bench

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Your DNA is constantly suffering damage, both from carcinogens like sunlight or cigarette smoke and from routine cellular process that cause DNA strands to break. If those breaks are not promptly repaired, they can give rise to cancer or birth defects — people with genetic mutations that affect their ability to repair DNA are at increased risk of cancer.

Stephen Kowalczykowski's laboratory in the Department of Microbiology, College of Biological Sciences studies how the DNA repair process works in organisms from bacteria to yeast and humans. Now, they have succeeded in reproducing a key step in repairing a broken double strand of DNA using purified proteins in a test tube.

"We've reconstituted one of the most complicated reactions in DNA break repair," said Kowalczkowski, distinguished professor of microbiology at UC Davis.

A DNA molecule consists of two strands that wind around each other in the famous double helix. The first step in repairing a break that crosses both strands is to partially unwind the broken end and strip away one of the strands, leaving a bare single strand, a bit like stripping a piece of wire.

Because every cell in an advanced organism like a human or yeast contains two copies of all the DNA on paired chromosomes, this bare end can be matched up with the complementary chromosome, which acts as a template to make an exact copy of the original.

Several genes are linked to faults in DNA repair. Working with yeast, the researchers tested the actions of the proteins made by these genes.

A complex of three proteins, called Dna2, Sgs1 and RPA, was sufficient to reproduce the unwinding and stripping step in a test tube. Of these, Sgs1 is a helicase that unwinds the DNA double helix; RPA (replication protein-A) binds to DNA, and also enhances Sgs1 activity; and Dna2 is a nuclease which digests away the unwanted strand of DNA.

The team also found two more active protein complexes. A combination of three proteins called Mre11, Rad50 and Xrs2, they found, acts as a signal. It binds to a broken end of double-stranded DNA and flags down passing Sgs1 to begin repairs.

Another protein, topoisomerase-3 (Top3), enhances the activity of Sgs1 by increasing its affinity for DNA. That is something of a surprise, Kowalczykowski said: Topoisomerases typically play a quite different role in managing the structure of DNA, for example by relaxing 'supercoils' in DNA.

Yeasts and people share similar genes for carrying out this form of DNA repair, although humans have more genes for some of these roles. The new paper also shows, for the first time, an analogy between eukaryotic organisms like yeasts and people, and prokaryotic bacteria. The "resection machine" of Dna2-Sgs1-RPA reproduces the functions of what Kowalczykowski calls his favorite protein, RecBCD, which carries out the same functions in E. coli bacteria.

The paper is published Sept. 2 in *Nature*. Coauthors on the paper are UC Davis postdocs Petr Cejka and Elda Cannavo; Piotr Polaczek, Taro Masuda-Sasa, Subhash Pokharel and Judith Campbell, all at the Division of Biology, California Institute of Technology. Funding was provided by the National Institutes of Health and the Swiss National Science Foundation, which provided a fellowship to Cejka.

This is the Kowalczykowski group's second paper in *Nature* in two weeks: on Aug. 22, they were one of two UC Davis labs to publish on the first purification of the BRCA2 protein, another protein linked to DNA repair and cancer. Here's our news release.