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Repair Protein Cradles Broken DNA

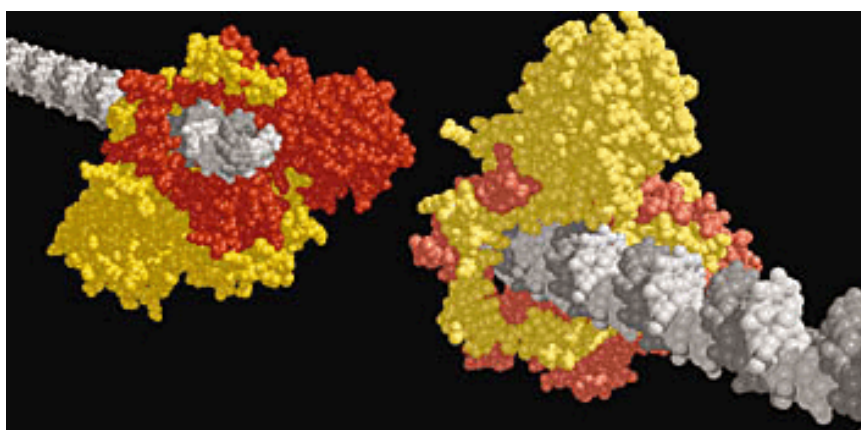


Image Title: A space-filling model showing Ku bound to DNA. Two components of Ku are colored red (Ku70) and yellow (Ku80). DNA is depicted with one dark gray and one light gray strand. - Jonathan Goldberg

Howard Hughes Medical Institute researchers have produced the first detailed images of a protein that performs the crucial task of detecting and repairing broken strands of DNA. The images show that the protein is constructed to cradle DNA while the DNA is repaired and rejoined with great precision.

The images of the DNA-repair protein Ku were published in the August 9, 2001, issue of *Nature* by Howard Hughes Medical Institute investigator Jonathan Goldberg and colleagues John R. Walker and Richard A. Corpina at Memorial Sloan-Kettering Cancer Center.

Breaks in double-stranded DNA can occur randomly as a result of exposure to ionizing radiation or as programmed events during the gene shuffling that is necessary to create infection-fighting lymphocytes. The Ku heterodimer, consisting of two subunits, Ku70 and Ku80, is a member of a family of DNA repair proteins that fixes damaged DNA in order to preserve the integrity of the genome. When Ku encounters damaged DNA, it initiates a repair process, called non-homologous end joining (NHEJ), which stitches double-stranded broken ends back together even though the ends of each DNA strand may not

be complementary.

Ku's role in maintaining the integrity of the genome was established by earlier studies in which HHMI investigator Frederick W. Alt and his colleagues knocked out Ku70 and other NHEJ components and found that DNA repair was compromised and that aberrant rearrangements of chromosomes occurred with high frequency. Although these studies reinforced the role of Ku in DNA repair, the details of how Ku senses and initiates repair were still sketchy. "The biochemistry is very clear," Goldberg said. "Ku is sitting in the nucleus ready to sense DNA damage and to bind to DNA ends."

What remained unclear, however, was how Ku was able to distinguish between broken ends and intact DNA with such precision. "Also, stitching DNA back together sounds dangerous because of the likelihood of losing genetic information," Goldberg said. "But actually, the non-homologous end joining process is quite accurate, and we wanted to find out why Ku appears to be so necessary for that accuracy."

Goldberg and his colleagues believed that seeing how Ku binds to DNA might provide answers to some of the questions about the Ku-DNA interaction. The researchers used a technique called x-ray crystallography to visualize the interplay between the Ku heterodimer and DNA. In x-ray crystallography, protein crystals are bombarded with intense x-ray beams. As the x-rays bounce off atoms in the crystal, they leave a diffraction pattern, which can then be analyzed to determine the three-dimensional structure of the protein.

Before they could get a full picture of the Ku-DNA complex, Goldberg and his colleagues decided to study the Ku heterodimer by itself. Their attempts to prepare crystals of the Ku protein yielded only a few usable crystals out of the hundreds they prepared. Fortunately, the scientists were able to use a technique pioneered by HHMI investigator Wayne Hendrickson to solve the complete structure of the Ku heterodimer from a single crystal. The technique, called multiple wavelength anomalous diffraction, was applied during crystallographic analyses performed at the National Synchrotron Light Source at Brookhaven National Laboratory. After the Ku structure was determined, the scientists moved on to solving the structure of the Ku-DNA complex.

In their studies, Goldberg and his colleagues had to mimic DNA breakage, ensuring that their test DNA fragment had only one accessible end — in order to avoid Ku attaching at more than one site on the DNA. They accomplished this by blocking the other end of the DNA with a bulky DNA motif.

After solving the structure of Ku bound to DNA, Goldberg and his colleagues could see how the Ku heterodimer manages to "find" a broken DNA end regardless of its sequence. "The problem is that Ku is not like a transcription factor that binds to a specific DNA sequence," said Goldberg. "Rather, it wants to recognize any broken DNA. And, the structure showed us that Ku is

a ring-shaped molecule that can slide onto the end as soon as the break is formed.”

The structure of the Ku-DNA complex reveals that the Ku heterodimer forms a ring that encircles and “cradles” the end of the strand of DNA. “We believe that the Ku proteins have to hold the DNA ends together,” said Goldberg. “The question is how they hold the end of a piece of DNA without obscuring the end. We found that our protein has an extensive base that cradles the DNA, with a very narrow bridge that lies over the top — holding one side of the DNA extensively, but leaving the other side almost completely exposed. We think this exposure might allow other repair factors to act on the broken ends to repair them.”

The scientists speculate that the Ku proteins on two broken ends link to one another to hold the two ends in position for joining the DNA back together. Goldberg and his colleagues also found that the Ku heterodimer makes no contact with the DNA bases, but rather grasps the sugar backbone of the DNA strand — meaning that the protein does not “care” about the sequence of the DNA that it binds. The scientists also have evidence that Ku holds the DNA in precise alignment to allow ready joining by repair enzymes. “It’s logical that if the protein precisely aligns the DNA ends, that gives an advantage to the repair proteins and the ligases that are going to ultimately join the DNA ends together,” said Goldberg.

Next, Goldberg and his colleagues plan to explore the structure of the Ku proteins attached to two broken strands of DNA in order to understand the mechanism by which they precisely align the ends. This precision is a key to the accuracy of the joining process in the absence of natural homology of the separated strands that could aid repair, Goldberg said.