

JUNE 15, 2000

DNA-Repair Machine Maintains Genomic Stability

The fidelity of the genome is under constant threat — toxic chemicals, ionizing radiation, even the byproducts of normal cellular metabolism can wreak havoc on DNA. In some instances, DNA damage is severe enough to chop the double helix in two. When this happens, a group of proteins that fixes double-stranded DNA breaks mobilizes to make repairs. If a break goes unfixed, the consequences to the cell can be disastrous, ranging from wholesale gene rearrangements to massive chromosomal breakdown.

Humans and other organisms are fortunate to have several repair systems that monitor DNA for signs of damage and initiate repairs when they are needed. HHMI investigator Frederick W. Alt and colleagues at Children's Hospital in Boston, Harvard Medical School and the Dana-Farber Cancer Institute are delving into these repair mechanisms and learning how the systems work and why they sometimes fail.

"This clearly shows that the end-joining pathway is extremely important in rejoining the ends of chromosomes damaged by ionizing radiation. If you don't have it, the cells are in big trouble."

— **Frederick W. Alt**

In a research article published in the June 2000 issue of *Proceedings of the National Academy of Sciences*, Alt and his colleagues report that mouse cells that lack a functioning nonhomologous DNA end-joining (NHEJ) pathway — which rejoins the broken ends of double-stranded DNA — show high levels of chromosome breakage and rearrangement of genetic material, called translocations. Such translocations are a frequent cause of leukemias and lymphomas. The take-home message from these studies, said Alt, is that the NHEJ repair pathway is critical to maintaining genomic stability.

In previous studies, Alt and his colleagues found that NHEJ is responsible for rejoining double-stranded DNA breaks in cells exposed to ionizing radiation and in lymphocytes that undergo gene shuffling during the process of creating infection-fighting cells. Those experiments revealed that mice

missing a key component of the NHEJ pathway suffered higher rates of B cell lymphoma, a cancer that is caused by the translocation of specific genes. So, Alt and his colleagues decided to see if NHEJ had a wider role in maintaining genomic stability.

"That finding of an increased rate of this particular translocation-related type of cancer led us to wonder whether a lack of end-joining could somehow predispose cells to genomic instability in general," said Alt. "If this were true, malfunctions in the NHEJ pathway might have more general implications for carcinogenesis."

The scientists examined embryonic mouse fibroblast cells that were deficient in one of three key enzymes (DNA-PKcs, Ku70 and Lig4) from the NHEJ repair pathway.

"We chose embryonic fibroblasts rather than tumor cells because we wanted to use cells that were as close to normal as possible," said Alt.

Using a new chromosome-imaging technique called spectral karyotyping, the researchers were able to spot the location of breaks in the otherwise featureless chromosomal landscape. In this procedure, researchers treat cells with fluorescent DNA probes that attach to specific DNA sequences on chromosomes. These colored probes paint each chromosome with a slightly different hue. A computerized imaging system attached to a microscope is then used to scan the cells. Looking through the microscope, a researcher can look for color differences to distinguish between the different chromosomes. The technique labels whole chromosomes and fragments of chromosomes, thus making the job of spotting broken pieces of DNA or chromosome rearrangements considerably easier.

"When David Ferguson used this detection technique, he found no chromosome abnormalities in the wild-type cells," said Alt. "When he looked at the NHEJ-defective cells, however, he saw a dramatic increase in chromosomal abnormalities in the cells that lacked Lig4 or Ku70. He also saw a smaller increase in abnormalities in the DNA-PKcs-deficient cells."

Alt and his colleagues also examined tissue directly from mice, noting the same kinds of abnormalities that they had seen in cultured cells.

In an experiment designed to assess the role of the NHEJ pathway in repairing general chromosomal damage induced by radiation, the scientists irradiated fibroblast cells that were deficient in both Lig4 and p53, a key DNA damage sensor. Normally, p53 prevents damaged cells from proceeding in the cell division cycle.

"We found a huge number of damaged chromosomes in the cells that were deficient in both Lig4 and p53 as compared to those deficient in only p53," said Alt. "Just about every chromosome had been broken to bits. This clearly shows that the end-joining pathway is extremely important in rejoining the ends of chromosomes damaged by ionizing radiation. If you don't have it, the cells are in big trouble."

Previous studies of chromosome repair suggested that a second repair pathway, the homologous recombination pathway, might be the major pathway responsible for accurate repair of damaged DNA because it seeks to match up DNA sequences when rejoining broken segments. Alt pointed out that this may not be the case, since his group's experiments show that translocations were infrequent in wild-type cells irradiated heavily enough to cause a high level of such damage in NHEJ-defective cells in which the homologous recombination pathway should be unaffected.

"These findings show that NHEJ really favors putting broken ends right back together, without going off and searching for breaks somewhere else," said Alt. "This capability adds to the evidence that the NHEJ is a crucial caretaker of the mammalian genome."