

APRIL 20, 2000

A New Genomic Caretaker

In every cell, a group of proteins called caretakers keeps vigil for signs of DNA damage. If damage is encountered, these proteins act immediately to repair the DNA and preserve the stability of the genome. Although small in number, these proteins perform a vital function and protect against potentially catastrophic consequences such as cancer-causing mutations or chromosome rearrangements, a frequent cause of leukemias and lymphomas.

In the April 20, 2000, issue of the journal *Nature*, HHMI investigator Frederick W. Alt and colleagues at the Children's Hospital in Boston provide evidence that XRCC4, a member of a family of proteins called non-homologous end-joining proteins, is a new type of genomic caretaker.

"Since translocation is a frequent mechanism underlying human lymphomas, understanding how the cells proliferate in this mouse system where lymphomas arise so frequently and reproducibly should give us insights into the mechanisms causing tumor generation."

— Frederick W. Alt

In previous studies, Alt and his colleagues found that XRCC4 helps to rejoin the broken ends of double-stranded DNA. Such breaks in DNA can occur randomly as a result of exposure to ionizing radiation or in the course of normal lymphocyte development. Alt's group found that XRCC4 was needed to rejoin DNA in lymphocytes that undergo gene shuffling in the process of creating the vast repertoire of infection-fighting cells. Curiously, the researchers also found that XRCC4 was needed to mend damaged DNA in the developing brains of mice.

"We had found that mice lacking XRCC4 died embryonically," said Alt, who is also on the faculty at Harvard Medical School. "When we looked closer, we found an expected defect in their lymphoid system, which wouldn't kill them, but we also found extensive cell death throughout their nervous system."

They reasoned that since the well-known genome guardian p53 monitors the cell for breaks in DNA, it was possible that p53 was "seeing" the unrepaired broken ends of DNA in the neurons and marking those cells for death.

To test this theory, Alt and his colleagues began with mice that lacked only one copy of *XRCC4*, and therefore survived past birth. By proceeding through multiple breeding steps using other mice lacking one or both copies of *p53*, they produced mouse strains completely lacking both *XRCC4* and either one or both copies of *p53*.

"Our educated guess proved out completely," said Alt. "When we got rid of *p53* in the *XRCC4* -deficient mice, they survived through embryonic development. Also, their nervous systems appeared largely intact, and they showed normal behaviors, such as the ability to walk and eat."

The scientists did note, however, that the lymphocytes in these mice did not show the normal gene rearrangement, called V(D)J recombination, needed to create the variety of lymphocytes necessary for the animals to fend off infection.

"We knew that B and T immune cells in these mutant mice can't put the immune gene segments back together, and without such recombination the cells can't develop past their progenitor stage," said Alt.

Importantly, he said, while the mice lacking both *XRCC4* and *p53* survived past birth, they went on to develop lymphomas at a high rate.

"A deficiency in DNA end-joining itself doesn't yield a high rate of tumors," explained Alt. "However, when we introduced this end-joining deficiency into a *p53* -deficient mouse, we did see frequent B cell lymphomas. We speculate that the lack of p53 allows those early progenitor lymphocytes with broken immune gene DNA to survive, and they give rise to tumors."

Particularly intriguing, said Alt, is that the B cell tumors arose largely from cells in which the recombination machinery had created a DNA break in the *IgH* gene locus and fused it to the *c-myc* gene, which is a well-known oncogene. As a result of the breakage and rearrangement, or translocation, of these genes, both genes become highly amplified, said Alt. The same type of rearrangement is also seen in the human cancer Burkitt's lymphoma.

"We don't yet know the reason for this translocation, whether there is a preferential breakage point, or whether there is a very strong selection for cells that are translocated at *c-myc*," said Alt.

Thus, the scientists' discovery of the interplay between p53 and XRCC4 may lead to a better understanding of how B cell lymphomas develop. "Since translocation is a frequent mechanism underlying human lymphomas, understanding how the cells proliferate in this mouse system where lymphomas arise so frequently and reproducibly should give us insights into the mechanisms causing tumor generation," he said.

The scientists will also explore how the absence of XRCC4 or other components of the DNA-repair machinery affects developing neurons.

"The implication is that these *XRCC4* -deficient mice have DNA breaks in their neurons that are not repaired, and p53 normally signals damaged neurons to die," said Alt. "But since, in the absence of p53, the end-joining deficient neurons still seem to function in a reasonable way, it doesn't appear that they need the end-joining process to put those DNA ends back together.

"So, we'd very much like to find out if there are any nervous system defects at all in the end-joining-deficient, *p53* -deficient mice, and whether the neurons in these animals retain broken ends that might give insight into the nature of the damage," said Alt.