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New DNA-Stitching Mechanism Important in Immune System

The immune system has a collection of strategies to help it fend off invaders - and researchers have just uncovered a new one. Scientists have long known that to generate the assortment of antibodies needed to recognize a diverse array of potential foes, immune cells shuffle segments of their genes into different combinations. Researchers have now found that in mending the DNA broken during this process, cells can employ a type of DNA repair that is fundamentally different than the classical method.

Howard Hughes Medical Institute investigator Frederick Alt and his colleagues published their discovery in the August 22, 2007, advance online publication of the journal *Nature*. First author was Catherine Yan in the Alt laboratory at The Children's Hospital and Harvard Medical School. The finding highlights the surprising importance of the alternate DNA repair mechanism in one of the most fundamental processes in the immune system, said the researchers. Also, they said, there are hints that the repair mechanism might play a role in the abnormal chromosomal rearrangements that underlie lymphomas.

The researchers explored how broken DNA is repaired in B cells, which are specialized immune cells that produce antibodies. Antibodies are proteins that recognize and attach to telltale components, called antigens, of foreign invaders such as viruses and bacteria, in order to destroy them. To generate specialized antibodies that can transport their antigen cargoes to specific destinations, B cells use a process called class switch recombination (CSR), in which genes for the antibodies are snipped and rearranged to produce different antibody types. The snipped-apart DNA must then be rejoined. Researchers have believed that B cells use a process called classical non-homologous end-joining for such repair.

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- **Frederick W. Alt**

To test whether this classical process is, indeed, involved in CSR, the researchers genetically knocked out two critical components of the end-joining machinery, proteins known as XRCC4 and Ligase 4.

Eliminating these proteins reduced the B cells' ability to rejoin DNA broken in CSR, suggesting that classical end-joining was important. But lots of class switching still occurred, and the researchers speculated that an alternate method of rejoining DNA ends must exist.

When they analyzed what kind of DNA breaks were repaired in the absence of CSR, they found that the alternate end-joining mechanism appeared to work only on pairs of broken DNA ends that had microhomologies. Microhomologies are short regions of overhanging single-stranded DNA that give the two broken DNA segments a complementarity—like two pieces of a puzzle fitting together.

"This finding tells us that something different is going on with this pathway that makes it strongly prefer microhomologies," said Alt. The next step, he said, is to explore exactly what characteristics of a DNA break trigger the cell to use the classical or alternate end-joining process. For example, he said, other researchers had found evidence that CSR tends to produce breaks with particularly long microhomologies, which might be preferentially repaired by the alternate process. "It may be that the two pathways are working in competition, and that the nature of the DNA substrates governs which one wins in each case," said Alt.

The researchers also plan studies to identify the components of the alternate end-joining machinery, said Alt. "While there could be some novel components, we think this pathway probably uses a subset of factors that are used for other DNA repair pathways," he said. "Once we have identified those components, we can dissect in normal cells what specialized role this repair pathway plays," he said.

Importantly, the Alt lab has generated evidence that the alternate pathway might have detrimental effects--catalyzing aberrant chromosomal

rearrangements, or translocations, that underlie lymphomas that arise in mice lacking components of the classical pathway and the p53 checkpoint protein.

“We don't really know if this is a 'bad' pathway,” said Alt. “However, our studies indicate that in the absence of the classical joining process, up to twenty percent of the breaks in CSR that do not get repaired get joined to other chromosomes as translocations,” said Alt. “So, for some reason the alternate pathway very effectively catalyzes translocations.” He also noted that studies done by others have indicated that the translocations that underlie many B cell lymphomas show microhomologies, which implies that they are being produced by the alternate pathway.

The alternative pathway had already been implicated from other studies but was not thought to function on chromosomal DNA. “Based on our current findings, this pathway will clearly be given more serious consideration, both for its ability to repair normal chromosomal DNA damage and for its ability to catalyze unwanted end-joins such as translocations,” said Alt.