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Ancient DNA-Repair Mechanism Helps Immune System Genetically Retarget Weapons

Researchers have learned how the immune system slices and dices genes so that B cells can program antibodies to seek out and destroy invaders. The new work suggests that an ancient DNA-repair mechanism that was designed to repair broken chromosomes may have evolved to play a key role in generating antibody diversity in the immune system.

Howard Hughes Medical Institute researcher Frederick Alt and his colleagues published their findings December 14, 2006, in *Science Express*, which provides rapid online publication of select articles from the journal *Science*. Ali Zarrin, who is in Alt's laboratory at Children's Hospital Boston and the CBR Institute for Biomedical Research at Harvard Medical School, was the first author of the article.

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B cells are the immune system's armories, where antibodies that attack viruses, bacteria and other invaders are produced. Before B cells are ready to take on specific pathogens, however, they must first tailor antibodies to recognize the invaders. To do this, B cells rearrange their antibody genes to make the highly variable, antigen-binding part of the antibody, through a process called V(D)J recombination. Later in development, B cells can also change the effector functions of antibodies that determine where antibodies go in the body and how they enlist the immune system's machinery to destroy their pathogen cargo.

To enable such programming, B cells undergo another type of gene rearrangement called class switch recombination (CSR). When undergoing CSR, B cells snip and recombine gene segments encoding the so-called constant region of antibody molecules that prescribes particular effector functions.

Alt and other researchers had shown previously that CSR requires two switch regions on the genomic immunoglobulin heavy (IgH) locus in order to work

properly. B lymphocytes secrete antibodies composed of IgH and Ig light (IgL) chains. One type of switch region is called the donor and it contains specific stretches of DNA that are to undergo CSR. The other is called the acceptor and it accepts the excised gene when it is snipped apart. This cutting and joining of the two widely separated switch regions allows one type of antibody constant region gene to be cut out and replaced with another. CSR also requires the DNA-altering enzyme, activation induced cytidine deaminase (AID). This enzyme attacks switch regions and generates lesions that lead to cuts in the chromosome referred to as DNA double-stranded breaks, which are critical in the process of rearranging the genes.

A major mystery was — once you had the breaks in switch regions, which are still separated by very long distances — why does the system join the donor switch region to an acceptor switch region? asked Alt. Why doesn't it just rejoin the breaks within one switch region and repair them right there?

We wanted to figure out whether there was special machinery specific to CSR to join the chromosomal switch region breaks that are so far apart or whether there was general double-stranded-break-repair machinery that that might put them together, said Alt. To understand the process in more detail, Zarrin set out to determine whether recombinational IgH class switching could still work even if he eliminated specific components. Zarrin removed the switch regions and AID and replaced them with very different types of components that could still generate chromosomal breaks in the IgH locus.

In an intricate set of experiments with mouse B cells, Zarrin replaced very large switch regions with short DNA sequences that would be recognized not by AID, but by a DNA-cutting enzyme from yeast called an endonuclease. When Zarrin introduced the yeast endonuclease into the mutant B cells with the replacements in place, IgH class switching still functioned, albeit at a lower-than-normal frequency.

We were quite surprised at what we found, as I believe were other people in the field, said Alt. This was a major piece of work on Ali's part, and to be quite honest, while I suggested this experiment, I thought it highly probable that it wouldn't work. Alt said that Zarrin designed his experiments so that he could independently determine whether either the switch regions or AID were required for recombinational class switching. In fact, neither was required for class switching in the mutant B cells, the experiments demonstrated, said Alt.

Zarrin also compared the relative ability of the yeast-enzyme version of IgH class switching to join both very close and distant DNA double-stranded breaks on the chromosome. He found that, while the close sites were joined at a higher frequency, the distant ones were still joined a significant amount of the time. This told us that the cells have a general mechanism that finds chromosomal breaks far away from one another and enables them to get joined, said Alt.

Thus, the findings with the altered B cells suggest that an existing general DNA repair mechanism that originally evolved to repair broken chromosomes has been adapted to join the widely separated DNA breaks

generated in CSR, said Alt. In this model, the switch regions evolved in B cells as targets for DNA-snipping AID which already existed in more primitive animals like fish where it serves to mutate Ig genes. Studies by Alt's laboratory and others have already implicated the normal double-stranded DNA break-repair machinery in CSR, he said.

The findings also have implications for understanding the types of chromosomal rearrangements that underlie some cancers. In his experiments, Zarrin found that breaks in the DNA created by AID could rejoin with other breaks on the same chromosome created by the yeast enzyme. This observation implies that the mechanism that produces breaks in CSR may not protect them from joining to other types of breaks. In contrast, noted Alt, the V(D)J rearrangement mechanism in B cells tightly controls chromosomal breaks, enabling them to rejoin only with similar types of breaks.

However, the mechanism that brings together distant breaks on a given chromosome still might actually protect against breaks on one chromosome joining with those on another — a classic occurrence in cancer-causing chromosome rearrangements. This mechanism might act as a sort of 'glue' to hold chromosomes together so that breaks are not allowed to migrate and be joined to other chromosomes, he said.