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Complex Gene-Swapping Spawns a Form of Lymphoma

Researchers have identified how a form of lymphoma develops when large pieces of chromosomes swap locations. Their observations about how chromosome rearrangements, or translocations, cause pro-B cell lymphoma in mice offer a fresh perspective on how cancers arise from an increased dosage of specific genes or regions of the genome.

The studies are reported in the June 28, 2002, issue of the journal *Cell* by Howard Hughes Medical Institute (HHMI) investigator Frederick W. Alt, HHMI associates Chengming Zhu and Kevin D. Mills, and colleagues at Children's Hospital in Boston, Harvard Medical School, the Center for Blood Research, and Brigham and Womens Hospital.

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— Frederick W. Alt

Leukemias and lymphomas can arise when chromosomes inappropriately swap genetic material. After the genetic material is swapped, various genes are removed from their natural context and they propel cell growth because they are no longer under normal control. In some cases, more complex gene translocations (as opposed to swapping one gene for another) lead to amplification of large regions of the genome. Alt and his colleagues coined the term *complicon*—derived from complex translocation—to describe this phenomenon.

Complicons may develop when the cells DNA repair mechanisms malfunction. When DNA repair fails, broken chromosomes can persist and recombine inappropriately with other chromosomes. This leads to chromosomal translocations—where genes or whole chunks of the genome are swapped from one chromosome to another.

Complicons are commonly seen in human carcinomas, in which very complex rearrangements cause two genes that are normally on different chromosomes to amplify together, said Alt. To date, however, there have been no mechanistic studies that have shown how complicons arise.

Alt and his colleagues, who have been studying a specific type of DNA repair process called non-homologous end joining (NHEJ), decided to probe whether DNA repair proteins are involved in complicon formation. They began by knocking out a major DNA repair mechanism in mice, effectively creating mice that lack one of two proteins central to NHEJ. Mice lacking NHEJ normally die before birth, because the cellular system for detecting high levels of DNA damage triggers programmed cell death, also called apoptosis. To keep the mice alive longer, the researchers also knocked out the gene for p53, which is an important component of the DNA damage detection system.

When we eliminated the p53 protein, the mice survived fine until after birth, said Alt. However, after only a few weeks they began to develop pro-B cell lymphomas, from which they died. Pro-B cell lymphomas are tumors that arise when cells of the immune system, called B cells, proliferate at an immature stage of development.

The researchers found that the lymphomas in these mice showed amplifications of genomic regions, or loci, containing two genes— *c-myc* and *IgH*—involved in B cell development. These amplifications somehow arose from complicons produced when the mouse chromosomes 12 and 15 underwent translocations.

To understand how complicons gave rise to these lymphomas, the scientists decided to test whether the translocations depended on a DNA-snipping enzyme called RAG. This enzyme is critical for joining and recombining genes in the normal development of B cells.

We thought a requirement for RAG was a good possibility, because we knew that the *IgH* gene locus was a target for RAG, said Alt. Also, it's been long proposed that RAG-induced chromosome breaks can lead to translocations that cause amplifications in other kinds of tumors.

The researchers demonstrated that RAG is required for complicon formation by making triple-knockout mice that lacked RAG, p53 and XRCC4, a protein involved in DNA repair. The triple-knockout mice failed to develop lymphomas. In addition, the researchers learned that the chromosome breaks involved in the lymphomas occurred where the RAG enzyme would normally snip apart DNA.

In a key set of experiments, the researchers used a technique called spectral karyotyping to analyze in detail the breakage and rearrangements of the chromosomes in the lymphomas. Basically, this technique traces chromosome rearrangements by painting individual chromosomes with fluorescent probes targeted to specific DNA sequences, said Alt. If you paint different chromosomes different colors, you can figure out where the

rearrangements came from.

Their analyses revealed that all the tumors showed a complex translocation—a complicon—that involved specific regions of chromosomes 12 and 15 that became embedded in a third chromosome. We found that the amplified *c-myc* genes, as well as parts of the *IgH* locus, were somewhere else, said Alt. They were on another chromosome that often had just pieces of chromosomes 12 and 15 attached. That was a surprise, because when we originally detected such a translocation between the two chromosomes, it resembled those of Burkitts lymphoma which just involve human equivalents of 12 and 15, so we thought that was where the action would be in this case; but it wasn't.

Furthermore, said Alt, the chromosome breakages that caused amplification of the genes were not found in a location where one might expect them to trigger *c-myc* activation. It was striking that every *c-myc* gene that we found to be amplified was located far away from the breakpoint, instead of adjacent to it, as is found in Burkitts lymphoma. The adjacent breakage classically damages the regulatory region upstream of the gene, switching it on, explained Alt. The mechanism that we have uncovered activates *c-myc* by leading to the amplification of the region of the chromosome in which it resides, he said.

In fact, Alt and colleagues demonstrated that the amplification mechanism leading to the complicons in the pro-B lymphomas is a breakage-fusion-bridge cycle similar to that proposed by Barbara McClintock in 1941 to explain unusual inheritance patterns in the corn plants she was studying.

The scientists analysis of the junctions between the rearranged chromosomes revealed that they were not stitched together by the normal DNA repair pathways. The researchers had already eliminated the NHEJ repair pathway in the mutant mice. In addition, the rejoining to produce the complicons did not use the DNA homologous recombination pathway, which looks for complementary strands on the two broken DNA ends. This repair pathway uses the complementary strands like splints to effect rejoining.

When we looked in the region of these junctions, there was no evidence of large homologies, said Alt. Instead, the complicons used another little-understood pathway that uses microhomologies of very short DNA sequences. This finding tells us that we must begin to focus on this pathway, since it can clearly catalyze these types of trans-chromosomal translocation events. HHMI investigator David Roth and his colleagues at Baylor College of Medicine discovered the microhomology-based end-joining pathway.

The discoveries by Alt and his colleagues should lead to a better understanding of how deficiencies in DNA repair and in the cellular DNA-damage-detection mechanism can produce tumor-causing complicons. The key point is that these types of cytogenetic lesions are seen in many human carcinomas, said Alt. Although there have been theories about their origin, until now nobody has shown mechanistically how they might occur.