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## Single Switch Triggers Two Immune System Genes

Two genes critical to the immune system's adaptability in battling viruses, bacteria and other invaders receive on and off signals from a single DNA segment, HHMI researchers have found. The discovery explains how the two genes work in concert and hints at how the genes have managed to remain partners for more than 450 million years since they first appeared in cartilaginous fish as part of an adaptive immune system.

In an article in the August 13, 1999, issue of the journal *Science*, HHMI investigator Michel Nussenzweig and colleagues at The Rockefeller University reported that neighboring genes, called *RAG1* and *RAG2*, are switched on in concert by a single genetic control signal nestled near *RAG2* on the chromosome. The discovery is like finding that a light switch in one house also controls lights in a house across the street.

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— Michel C. Nussenzweig

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The *RAG1* and *RAG2* genes produce proteins that somehow join to form a "transposase," an enzyme that helps snip apart and rearrange genes that code for two critical weapons in the immune system's arsenal. These weapons are protein antibodies produced by the immune system's B cells and receptors found on the surface of T cells.

Antibodies are molecules that roam the bloodstream, recognizing and attaching to invaders and marking them for destruction. Similarly, the receptors on T cells—the immune system's infection-fighting "soldiers"—are those that recognize antigens, fragments of foreign proteins, so that the T cells can attack the viral or bacterial proteins.

The *RAG1/RAG2* recombinases' ability to rearrange the genes for such antibodies and receptors is crucial to the immune system's flexibility in

generating a nearly infinite variety of weapons against infections and malignant cells. HHMI investigator David Schatz discovered RAG1 and RAG2 in 1989, and in 1998 with colleagues at Yale University, he showed that RAG1 and RAG2 had transposase activity.

"Before our studies, we knew that the two genes were controlled coordinately," said Nussenzweig. "However, we knew very little about their control elements." He added that previous experiments testing small segments of DNA were inconclusive in defining the *RAG1/RAG2* control mechanism.

In the current work, however, Nussenzweig and his colleagues used specially modified bacterial artificial chromosomes (BACs) to carry large pieces of DNA into mouse cells. HHMI investigator Nathaniel Heintz, also at Rockefeller, invented the BAC modification technique.

Using BAC to shuttle DNA into the mice, the researchers created transgenic mice that possessed fluorescently-tagged *RAG* genes and extended DNA segments adjacent to the *RAG* genes that the researchers suspected carried the *RAG* controller. In addition, Nussenzweig's team used the BACs to introduce genes coding for two fluorescent proteins—one that would shine green wherever RAG1 appeared, the other yellow in cells expressing RAG2.

By inserting such test segments into mice and looking for fluorescent B and T cells, the scientists could determine whether those segments contained a control sequence that properly switched on the *RAG* genes.

"We used the marker genes to show us where the RAGs were being expressed, and then we used recombination technology to knock off pieces of the bacterial artificial chromosomes to reveal where the control elements might be," explained Nussenzweig.

After many such experiments with various constructed segments, the scientists narrowed down the control sequence to a small piece of DNA next to one end of the *RAG2* gene.

The discovery that the neighboring *RAG* genes are controlled by a single switch has important implications for understanding how the two genes evolved as part of the immune system, said Nussenzweig.

"The configuration of these genes makes scientists believe that they were inserted into the genome together, as a 'transposable element,'" he said. What's more, said Nussenzweig, the finding may explain why, over the 450 million years since the genes first appeared, evolution has required them to remain closely spaced in the genome.

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The key to the genes' long-lived marriage might lie in the mechanism by which the control sequence activates both genes at once, said Nussenzweig. In the *Science* paper, he and his colleagues propose two possibilities: First, the control element may bounce back and forth between the promoter regions of the two genes and activate them; alternatively, the controller may touch both simultaneously.

Although Nussenzweig emphasizes that there are no data that reveal which is the correct mechanism, the theory of simultaneous activation might better explain why evolution has refused to allow the two genes to separate and still remain functional partners in the transposase enzyme. He and his colleagues plan experiments that they hope will reveal the control mechanism for RAG1 and RAG2.