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Cell Silences Genes on the Nuclear Edge

In the cell's nucleus, neighborhoods matter. By corraling certain genes at the edge of the nucleus, developing immune cells can control a gene's activity and keep it switched off, according to a new study from Howard Hughes Medical Institute (HHMI) researchers.

The finding comes as the result of researchers' efforts to understand how blood stem cells mature into the various types of immune system cells that recognize invading bacteria and viruses. Senior author Harinder Singh, an HHMI investigator at the University of Chicago, and his colleagues reported their finding February 13, 2008, in an advance online publication of the journal *Nature*.

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— Harinder Singh

The human immune system relies on several types of cells to recognize foreign invaders so that the body can destroy them. Two of these, known as B cells and T cells, can recognize myriad invaders and help coordinate a customized immune response. Receptors that stud the surfaces of B and T cells aid this recognition process by binding to antigen molecules on the surface of the invading pathogen. In B cells these receptors are called antibodies; T cells have different structures called T cell receptors.

Both B cells and T cells mature from blood stem cells, and use the same set of proteins to organize activation of their receptor genes. But the set of genes that encodes B cell antibodies is different from the set of genes that encodes T cell receptors. One main question has intrigued Singh: Given their developmental similarities, how do B and T cells avoid turning on the wrong genes?

Singh's previous work suggested that nuclear neighborhoods might provide part of the answer. In blood stem cells, the gene segments that encode both

antibodies and T cell receptors are attached to the inside of the nuclear membrane. Singh's group had found that the antibody genes were being moved [toward the center of] the nucleus in B cells as they developed, said Singh. In developing T cells, on the other hand, those same gene segments—which are never switched on—remained stuck to the membrane.

Singh wondered if this organization created miniscule nuclear neighborhoods whose genetic occupants could not be activated. If so, Singh reasoned that developing T cells might leave the unneeded antibody genes pinned against the nuclear membrane to avoid the potential for mistakes. To test the idea, he said, We needed to develop a method where we could move a gene—any gene—within the nucleus.

Their goal was to put a gene on the outskirts of the nucleus, next to the nuclear membrane. And in the experiments reported in *Nature*, Singh, his postdoctoral fellow Karen Reddy, and their colleagues tricked mouse cells into doing just that. The team attached a binding site for the protein LacI to a gene that makes a cell resistant to the cell-killing drug hygromycin, and inserted the assembly into a mouse cell. The team chose the hygromycin resistance gene because it provided any easy way to test gene activity; when exposed to the drug, only cells with an active gene would survive.

In the same cells, they also activated LacI, which dutifully latched on. But in a bit of scientific trickery, the researchers first fused LacI to two other proteins. The first was green fluorescent protein (GFP), which glows brightly and allowed the team to identify the assembly's location. The second was a protein called emerin, which normally resides in the nuclear membrane. Regardless of the other proteins and DNA that the researchers had attached to it, the cell recognized that emerin belonged in the membrane, and dragged it there, Singh explained. When it did, the attached test gene stopped functioning, as did neighboring genes.

What we have been able to show is that location or neighborhood of a gene within the nucleus actually matters in terms of its activity, said Singh. It's evidence. . . that this sort of repositioning has consequences.

Besides answering why developing immune cells sequester genes at the edge of their nuclei, Singh said the study opens intriguing new avenues for research. According to Singh, the mechanism only works in cells that are still dividing. When we block cells from dividing, we cannot drag these genes to the inner nuclear membrane, he said. Singh thinks these nuclear neighborhoods might help specialized cells in the muscles, nerves, and organs customize which genes they express as they develop from non-specific stem cells. We think that this sort of mechanism might be used quite widely in the body, he said.

Singh also said that the mechanism might one day be used in treating diseases caused by malfunctioning genes. With a better understanding of the molecular mechanism that cells use to position genes, Singh said, scientists may begin to be able to develop drugs that take advantage of this process, he said. In the context of cancerous cells, if you want to turn genes off that are

inappropriately active and driving that cancer, one way to do it might be to drag them to the nuclear membrane. However, he said, I think [such treatments are] quite a ways away.