Key Piece Found for Immunology Puzzle?

Researchers may have found the gene for the long-sought enzyme that assembles antibody and T cell receptor genes

ALTHOUGH KNOWLEDGE OF THE IMMUNE SYSTEM has increased dramatically in recent years, researchers have been stymied for over a decade in their quest for one key piece of the puzzle—until perhaps now. Back in the late 1970s, scientists discovered that the genes for antibody proteins are assembled from several separate segments of DNA. That finding helped to explain how the immune system can generate the vast number of different antibodies needed to fight off infections. But it raised another question: What enzyme joins the DNA segments that make up an antibody gene?

Now researchers from the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, may have put their fingers on the missing piece of the puzzle. In the 22 December issue of *Cell*, David Schatz, Marjorie Oettinger, and David Baltimore report finding a gene that may encode the long-sought enzyme, which goes by the name "recombinase."

The Whitehead researchers are the first to admit that they have not yet proven that it does. Indeed, there is at least one other good recombinase gene candidate. But at the very least, the gene found by Baltimore and his colleagues encodes a regulatory protein that turns on the actual recombinase gene. "Either way it's marvelously interesting," says immunologist Martin Gellert of the National Institute of Diabetes and Digestive and Kidney Diseases. "It's the best thing that's happened in the molecular biology of that [gene-assembly] system so far. We knew what went in and what came out, but we didn't know how it happened."

Immunologists are so interested in the recombinase because the immune system simply will not work without the gene rearrangements that it brings about. Not only are they necessary for the maturation of the antibody-producing B cells, but they are also required for the development of the immune system's T cells, which kill virus-infected cells and regulate other immune cell activities. Receptors on the surface of the T cells enable them to interact with their target cells, and the genes for the receptors are assembled from three or four separate DNA segments—much as antibody genes are.

Without the recombinase, the body would lack functional B cells and T cells and would be unable to defend itself against infection. Defects in the recombinase system might therefore cause inherited immune deficiencies such as the one that afflicted the "boy in the bubble," whose plight was portrayed in a television movie a few years ago. He had to live in a germ-free environment because his immune responses were defective. The recombinase is unlikely to be involved in AIDS, however, because the AIDS virus infects mature T cells long after the time the recombinase was active.

Schatz, who has been a graduate student in Baltimore's lab for the past 5 years, says that the key to the group's success in isolating the candidate recombinase gene was the development of an assay to detect gene rearrangements like those that form the antibody and T cell receptor genes. To perform the assay the researchers first introduce into mouse fibroblasts a hybrid DNA that will serve as a gene-rearrangement indicator. The hybrid DNA contains antibody gene segments that can be joined by a recombinase, along with a gene for antibiotic resistance that is activated only when the rearrangement takes place.

Normally fibroblasts cannot carry out antibody gene assembly and they die when exposed to the antibiotic. But when the researchers introduced either human or mouse genomic DNA into the fibroblasts, they got a different result. "After you put the genomic DNA into them, you can find the rare cell that becomes drug-resistant because it carries out the rearrangement," Schatz says. The Whitehead group has now isolated both the human and mouse genes that conferred that ability on the fibroblasts. The two genes are almost identical and have been given the designation RAG-1, for recombination-activating gene 1.

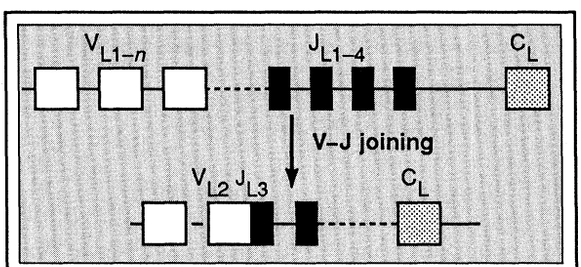
The researchers can't yet tell for sure whether RAG-1 produces the gene rearrangement in fibroblasts because it encodes the recombinase itself or because its product turns on the fibroblast gene that does code for the enzyme. But there is circumstantial evidence that RAG-1 is the recombinase gene. The gene's structure has been well maintained in evolution, as have other aspects of the gene assembly system for antibody and T cell receptor proteins.

In addition, the RAG-1 gene is turned on only in T and B cells that have recombinase activity. "This doesn't prove that what we've found is the recombinase," Oettinger remarks, "but it's certainly what we expected to see."

However, Melvin Bosma's group at the Fox Chase Center for Cancer Research in Philadelphia has identified another strong candidate for a recombinase gene. This gene, designated *scid* (for severe combined immunodeficiency), is mutated in mice with a severe hereditary immune deficiency. According to Michael Lieber of Stanford University School of Medicine, the lymphocytes of *scid* mutants cannot carry out normal antibody gene rearrangements.

At first the Whitehead researchers thought RAG-1 and the *scid* gene might be the same, but subsequent work showed that they are not; they are located on different chromosomes. And yet there may be some relationship between RAG-1 and the *scid* gene. Schatz, Oettinger, and their colleagues are trying to find out if the RAG-1 protein has recombinase activity of its own. If it does, then the *scid* product is probably an accessory to that enzyme. But if it doesn't, then the *scid* gene may encode the recombinase, with the RAG-1 product acting to control the *scid* gene.

Right now Lieber says his bet would be on the first possibility—that RAG-1 is the recombinase gene. But even if RAG-1 only encodes a recombinase activator, it would still be important as a key regulator of T and B cell development. ■ JEAN MARX



Antibody gene assembly. *Three gene segments, designated V (for variable), J (for joining), and C (for constant) are joined to make the smaller of the two proteins that form antibodies. The genes encoding the larger antibody protein have a fourth segment, called D (for diversity), interposed between the V and J segments. The recombinase brings about the V-J and V-D-J joining early in B cell development.*