The Accidental Immune System

Long ago, a wandering piece of DNA—perhaps from a microbe—created a key strategy

By JOHN TRAVIS

Immunologists have wrestled with the origin of GOD for several decades.

This spiritual-sounding acronym stands for "generation of diversity" and emerged in the jargon of scientists after their realization in the 1970s that the human immune system manufactures tens of millions of distinct antibodies. These elaborate, Y-shaped molecules latch onto and help destroy infectious microorganisms. Since the human genome seems to contain fewer than 100,000 genes, the remarkable variety of antibodies initially baffled researchers. How could so few genes produce so many antibodies?

In 1976, scientists proved that this remarkable antibody diversity stems in large part from the ability of antibody-producing immune cells, called B cells, to shuffle pieces of their genes. By fusing various combinations of gene segments, a maturing B cell generates unique DNA sequences encoding parts of the antibody's binding region. Although they don't make antibodies, other immune cells use this same shuffling mechanism to create on their surfaces similar microbe-binding proteins called T cell receptors.

Curiously, while animals ranging from sharks to people use this so-called combinatorial strategy, more primitive organisms such as starfish, lampreys, and horseshoe crabs lack it. As a result, they don't make typical antibodies or T cell receptors. "The combinatorial immune system is found in all jawed vertebrates but, to the best of anybody's knowledge, nowhere else," says John J. Marchalonis of the University of Arizona in Tucson.

Marchalonis and his colleagues have taken to calling the emergence of this system the "big bang" of immunology, especially since scientists estimate it appeared within a span of just 20 million years, a blink of the eye in evolution's time frame. When scientists first grasped the explosive nature of this evolutionary event, they were at a loss to explain how it could occur. "We didn't have a mechanism," says Marchalonis.

In recent years, researchers have crafted a surprising explanation for this immunological advance. Hundreds of millions of years ago, in a creature perhaps resembling today's sharks, a piece of DNA apparently jumped from its chromosomal home, a movement known as transposition. Carrying the blueprint for an enzyme that cuts and pastes DNA, it just happened to insert itself within a gene, perhaps one already being used by the immune system. Over time, this fragment endowed descendants of that ancient creature with the DNA-shuffling ability needed to create vast arrays of antibodies and T cell receptors.

"One might argue that such complex organisms as mammals (and other vertebrates) can only exist thanks to an immune defence system whose repertoire matches that of invading viruses and microorganisms. . . . If that is true, we may owe our existence to one transposition event that occurred 450 million years ago," immunologist Ronald Plasterk of the Netherlands Cancer Institute in Amsterdam says in the Aug. 20 NATURE.

Even more startling, scientists suspect that this fortuitous piece of mobile DNA originally came from a bacterium, virus, or some other infectious microorganism. In other words, the most sophisticated part of the human immune system likely arose from an infection by one of the enemies it now fights.

People and most other vertebrates actually have two immune systems. The first is the innate system, which is made up of cells and molecules that recognize and bind to characteristic features of microbes, such as standard bacterial cell wall components. The innate immune system gives a rapid response to most microbial invasions. Yet, the nonspecific weapons employed can have distressing side effects on the body. Inflammation, for example, is largely a by-product of the innate immune response.

The combinatorial immune response, also known as the adaptive or anticipatory response, represents the second vertebrate immune system. Of the many millions of antibodies and T cell receptors generated by it, a select few will recognize the unique molecular features, or antigens, of particular infectious agents.

Antibodies might bind to knobs or other features found only on the surface of a certain flu virus, for example. Over several days, the immune system then tailors its response to the selected antigens by mass-producing the relevant antibodies and T cells. The next time the body confronts the same antigens, the appropriate antibodies and T cells muster more quickly. This immunological memory explains the power of vaccines.

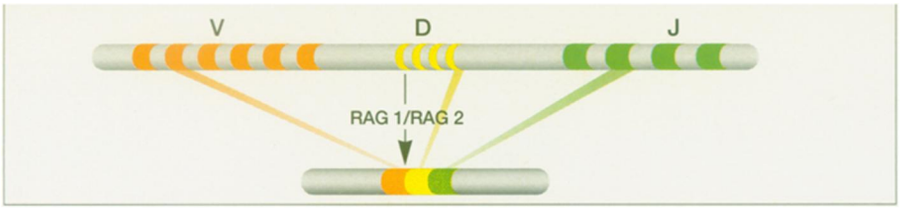
Biologists have suggested that the development of the combinatorial immune system allowed animals to live longer, but this doesn't account for creatures such as horseshoe crabs that survive for decades with just their innate immune response. What good is the diversity of antibodies and T cell receptors?

"You don't have to devote as many resources to an infection problem if you can quickly make the immune response specific," speculates Craig B. Thompson, a Howard Hughes Medical Institute investigator at the University of Chicago. "I think it's an advantage of efficiency and focus." Conserving energy would have been vital to primitive animals struggling to survive hundreds of millions of years ago, he adds.

Whatever the original evolutionary advantage, the human species has grown dependent upon the combinatorial immune system. Mutations that prevent proper antibody and T cell–receptor formation are usually fatal unless people are isolated to avoid infections.

To create a vast repertoire of antibodies and T cell receptors, maturing immune cells rearrange DNA as effortlessly as dealers in Las Vegas shuffle cards. This immune system shuffling is needed because the genes encoding antigen-binding sites come in pieces, which are known as V (for variable), D (for diversity), and J (for joining). The rearrangement is therefore known as VDJ recombination.

There may be dozens to hundreds of V, D, and J gene segments in any immature B cell or T cell. As they mature, the cells randomly cut out and discard all but one segment of each class. They then fuse the remaining segments together to form the working gene. The final fusion contributes to the variation because an identical array of V, D, and J segments can reconnect in subtly different ways. "The system is deliberately imprecise," says immunologist Martin Gellert of the National Institute of Diabetes and Digestive and Kidney Dis-



To make the genes for key parts of an antibody, the proteins RAG1 and RAG2 help recombine V, D, and J gene segments after removing the other available segments.

eases in Bethesda, Md.

Once scientists recognized VDJ recombination, a discovery that earned a Nobel prize, they began to wonder how cells accomplish it. Suspicion immediately focused on odd stretches of DNA called transposons, or transposable elements, that have a well-known ability to cut and paste genetic material.

First discovered in maize and now observed in most genomes, these mobile stretches of DNA encode an enzyme, called a transposase, that snips DNA at the beginning and end of the transposon. The excised transposon is then free to move to another spot on a chromosome and insert itself there with the help of the transposase.

"The element is completely autonomous. It can jump from place to place to place," says David G. Schatz, a Howard Hughes Medical Institute researcher at Yale University School of Medicine.

Scientists studying VDJ recombination noticed that flanking each V, D, and J gene segment were brief DNA sequences that resemble those on which transposases operate. From that initial hint grew the idea that immune cells had somehow coopted a viral or bacterial transposase to cut out unnecessary V, D, and J segments—but not insert them elsewhere.

The transposon connection grew stronger in around 1989 when Schatz and his colleagues isolated two genes that drive VDJ recombination. These recombination-activating genes, known as *RAG1* and *RAG2,* sit right next to each other, suggesting they were once packaged together as a transposase, says Schatz.

RAG1 and RAG2, the proteins encoded by the two genes, initiate VDJ recombination by binding to the specific DNA sequences that flank V, D, and J segments and cutting the DNA at those sites. The excised DNA is removed and DNA-repair enzymes, aided in a some manner by RAG1 and RAG2, rejoin the remaining V, D, and J segments.

RAG1 and RAG2 cut strands of DNA in a way scientists found notable. "The chemistry of the reaction looks like that of a transposable element," says Gellert.

Yet, scientists weren't convinced that RAG1 and RAG2 together make up a complete transposase, in large part because the proteins don't now seem to reinsert the clipped DNA into another stretch of DNA, as the enzymes normally do.

"Even if they were a transposase [millions of years ago], there was a lot of skepticism that they still had that function," notes Schatz.

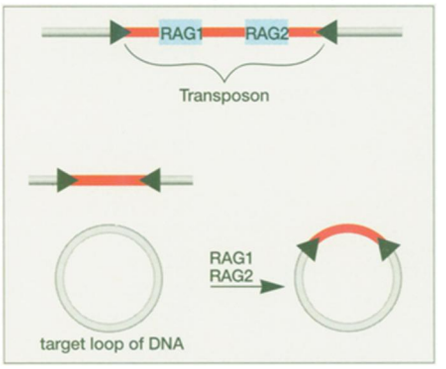
So, both his group and Gellert's set out to test the proteins' skills. Last year, for example, Gellert and his colleagues showed that the RAG proteins could, in test-tube experiments, actually join DNA strands, a requirement for a fully functional transposase.

In the Aug. 20 NATURE and Aug. 21 CELL, the two research groups show that the pair of proteins can perform a complete transposition reaction in a cell-free system. Both groups mixed together RAG1, RAG2, a DNA sequence nestled between the recombination sequences recognized by the two proteins, and a loop of target DNA. The investigators then tested whether the proteins were able to snip out the flanked DNA sequence and add it to the target loop.

An increase in the molecular weight of that circular DNA strand provided clear proof that RAG1 and RAG2 had all the skills of a true transposase. "In the test tube, they perform the transposition reaction with gusto," says Schatz.

This new work is "wonderful" and a "very satisfying conclusion to a 22-year-old problem," says Thompson, who in 1995 provided a detailed theory about how a transposon might have created VDJ recombination.

Researchers now hope to determine whether RAG1 and RAG2 can carry out their cut-and-paste activities inside living cells. As a protective measure, modern cells may have developed means to suppress the ability of RAG1 and RAG2 to reinsert the DNA sequences they remove.



Scientists believe the genes for RAG1 and RAG2 were once part of a mobile DNA sequence called a transposon (top). The arrows on the DNA sequence mark where the RAG proteins would cut the DNA to allow the transposon to move. Recent experiments (bottom) show that RAG1 and RAG2 retain their ability to cut and paste DNA.

"Vertebrates seem to have tamed this ancient transposon," notes Plasterk in his NATURE commentary.

Or have they? The cell's efforts seem to fail sometimes. In their CELL paper, Gellert and his colleagues contend that transposition events mediated by the RAG proteins actually trigger lymphomas and leukemias, cancers of immune cells. The scientists suggest that if the excised DNA joins an oncogene—a cancer-promoting gene—in just the right way, the event could turn on the oncogene full force. This would stimulate the uncontrolled cell division that characterizes cancer. Such transpositions "may explain a fair number of these [cancers] that were kind of mysterious," says Gellert.

While the cancer issue intrigues Schatz, he bets that cells can largely prevent these potentially damaging transposition events. A suppressive mechanism has not yet revealed itself, however. "We're hopeful we'll be able to figure it out," says Schatz. He and Gellert would also like to understand in more detail how RAG1 and RAG2 together perform VDJ recombination and, in the test-tube system at least, both cut and paste DNA.

Scientists continue to wonder where the transposon that originally employed RAG1 and RAG2 came from. Since bacteria, viruses, and other infectious organisms have enzymes that cut and paste DNA, they suspect that a microbial infection—one that made it deep enough into the body to infect a sperm or egg—transferred the mobile element into an ancient jawed vertebrate.

Presumably, the transposon hopped around the genome until it landed in the middle of a gene for an ancestral antibody or T cell receptor, breaking the gene into pieces that only RAG1 and RAG2 could reassemble. Gene duplication and other evolutionary changes would then have created the combinatorial immune system from that original transposition event.

Investigators have searched vigorously for similarities among the shapes of the RAG proteins and known microbial enzymes, but they've only found the slightest resemblances, and even those seem contradictory. Some point to a virus like HIV, which causes AIDS, as the source of the transposon, while others point to bacteria or fungi.

"We might never find where it came from," says Gellert, noting that the responsible microorganism might be extinct.

So, was the creation of the combinatorial immune system merely a matter of incredible luck? Perhaps. Another legitimate view, argues Schatz, is that considering the millions of years, millions of creatures, and millions of transposition events that evolution has had to work with, something like this was bound to happen. □