Antibody Research Garners Nobel Prize

A molecular biologist is honored for his contributions to understanding how the immune system makes such a vast repertoire of antibodies

SUSUMU Tonegawa of the Massachusetts Institute of Technology (MIT) has won the 1987 Nobel Prize for Physiology or Medicine for his contributions toward understanding how the body makes the multitude of antibodies that it needs to fight off disease. The work helped to resolve an immunological puzzle of long standing—namely, the antibody diversity question, which Thomas Waldmann of the National Cancer Institute describes as the "preeminent immunology problem over the past three decades."

The question concerns how the cells of the body can possibly contain enough genetic information to make the essentially unlimited number of antibodies that are required to combat all the foreign invaders, including pathogenic viruses and bacteria, that an individual might encounter during a lifetime.

Beginning in the mid-1970s, Tonegawa, who was then working at the Basel Institute for Immunology in Switzerland, showed that much of the diversity was caused by assembling the genes encoding the antibody proteins from three or four separate segments of DNA. "Tonegawa discovered something largely unprecedented," Waldmann explains. "You make this large array of molecules by DNA rearrangements, the way you would use the letters of the alphabet to generate words."

For years, immunologists had debated two competing explanations of antibody diversity. The "germline" theory, as it was called, proposed that all the genes required for making antibody proteins are already formed in the germ cells, the sperm and egg. The problem was that the genome is not big enough to hold the large number of genes that this theory predicts, as well as all the others needed to build an animal.

In contrast, the "somatic mutation" theory proposed that only a limited number of antibody genes exist, but that these mutate readily during the development of the antibody-producing cells to yield numerous antibody variants.

Then in 1965, William Dreyer of the California Institute of Technology and J. C. Bennett of the University of Alabama School of Medicine suggested a way of encoding antibody molecules that would reduce the amount of DNA required. A complete antibody molecule consists of two heavy protein chains and two smaller, light chains. Analysis of the amino acid sequences of these proteins showed that each could be subdivided into a constant region, which is the same for all chains of a particular type, and a variable region that changes from one chain to the next. The variable regions of the light and heavy chains combine to form the portion of an antibody that recognizes and binds foreign antigens.

Dreyer and Bennett's suggestion, encapsulated as the "two gene–one polypeptide theory" of antibody synthesis, was that the variable and constant regions of a given antibody protein might be encoded by separate DNA segments that could be combined. That way there would only have to be one copy of the constant region gene, although there still might be many variable region genes, for each antibody chain.

In 1976, Tonegawa and his Basel colleague Nobumichi Hozumi reported the first direct evidence that the variable and constant regions of an antibody light chain are encoded separately and then joined, in agreement with the proposal of Bennett and Dreyer. Tonegawa and Hozumi examined DNA from mouse embryos and from a line of mouse tumor cells derived from antibody-producing B lymphocytes. They found that the genes encoding the variable and constant regions of the protein were much farther apart in the embryonic DNA than in that from the antibody-producing cells. "That experiment showed that the gene underwent rearrangement," notes Philip Leder of Harvard Medical School.

The research on the antibody diversity problem was greatly aided by the ability, newly acquired in the 1970s, to clone genes and to determine their nucleotide sequences. Tonegawa, in collaboration with Walter Gilbert and Allan Maxam at Harvard University, went on to sequence a light-chain gene from the tumor cells.

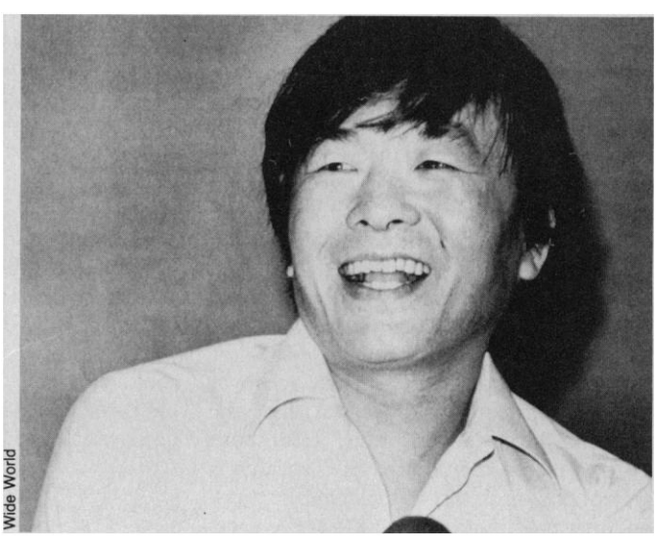
The results provided a surprise. Although the original experiment of Tonegawa and Hozumi had indicated that the variable and constant gene segments, which are designated V and C, had come together in the tumor cells, the sequence data showed that the two gene segments are still separated by 1250 base pairs of DNA that do not code for light-chain protein. "V and C are close together," Tonegawa says, "but they are not contiguous."

The antibody gene was one of the first mammalian genes found to be interrupted by a noncoding DNA sequence. Researchers soon learned that these introns, as they came to be called, are common features of the genes of higher organisms.

Tonegawa and his colleagues encountered a second surprise when they determined the nucleotide sequence of a fragment of embryonic DNA that contained the variable gene for the light chain. They found that the V gene terminated after the codon for the 98th amino acid of the antibody protein. Variable regions contain approximately 110 amino

Susumu Tonegawa

At an MIT press conference that was held after he was awarded the Nobel Prize for his work on antibody genes.



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acids, and the gene was therefore too short to encode the entire region.

Leder, who was then at the National Institute of Child Health and Human Development, and his colleagues had sequenced the gene for another light-chain variable region, and had come to a similar conclusion. A gene segment encoding some 13 amino acids of the variable region was missing, a finding that raised the possibility that two separate DNA segments might be needed to encode a light-chain variable region. This possibility received additional support from comparisons of the amino acid sequences of light chains that were performed by several investigators, including Leroy Hood of Caltech and Martin Weigert of the Fox Chase Institute for Cancer Research in Philadelphia.

Tonegawa and his colleagues soon tracked down the missing DNA segment, which was designated J for joining. In embryonic DNA, it is located 1250 base pairs—the exact size of the intron in the gene from mature antibody-producing cells—before the start of the constant region gene. "The J sequence was upstream of C," Tonegawa explains. "We could then reconstruct what was happening with respect to V-J joining."

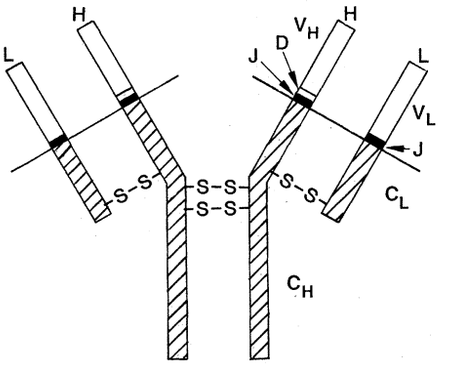
According to the picture that emerged, the V and J segments, which are widely separated in embryonic DNA, become joined during the maturation of an antibody-producing B cell to form the complete coding sequence for a light-chain variable region. This coding sequence remains separated from that for the constant region, however, by an intron, which is not cut out until after the whole stretch of DNA is transcribed into messenger RNA.

The assembly of the genes for antibody heavy chains takes place in an analogous manner, except that Tonegawa's group and also that of Hood found that three separate DNA segments must be joined to form a complete coding sequence for a heavy-chain variable region. The third coding segment, which was designated D for diversity, lies between the V and J segments.

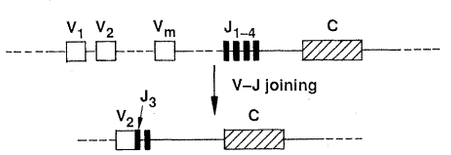
The V region variants that can be used to construct heavy and light chains number in the hundreds, and there are also several J, or J and D, variants for most antibody chains. Since any V-J or V-D-J combination is apparently possible, this means that genetic rearrangements can produce tens of thousands of both light and heavy chains. Then, since any light chain may associate with any heavy chain to form an antibody molecule, the total number of combinations is conservatively in the hundreds of millions.

Moreover, although the DNA sequences flanking the V, D, and J elements help to ensure that they make the right connections—V with J, for example, and not with another V—the joinings are not always exactly accurate, a circumstance that introduces still more variability into antibody proteins. Finally, work by Hood, among others, showed that mutations also occur in antibody genes, in accordance with the old somatic mutation theory.

By the beginning of the 1980s, the work of Tonegawa and the other investigators had delineated the basic principles of antibody synthesis, showing it to be an elegant system in which gene rearrangements, multiple copies of gene segments, and somatic mutations all contribute to the generation of antibody diversity.



Antibody molecule structure. *The two heavy chains (designated by H) and the two light chains (L) are held together by disulfide bonds, as indicated. The variable regions of the light and heavy chains (shown above the lines) form the recognition sites that bind foreign antigens; there are two binding sites per molecule. The two sections of the light-chain variable region, designated V_L and J, are encoded by separate gene segments, as are the V_H, D, and J sections of the heavy-chain variable region.*



Light-chain gene assembly. *The upper diagram shows the arrangement in embryonic cells of the three gene segments (V for variable, J for joining, and C for constant) that are needed to make an antibody light chain. At this stage of development, the V and J gene segments are far apart. During the maturation of antibody-producing cells, any of the many V segments can be joined to any J segment. The variable sequence thus assembled remains separate from the C sequence until after the whole stretch of DNA is transcribed into messenger RNA. Then the intervening sequence is cut out, and the messenger RNA is translated into a light-chain protein.*

One unexpected consequence of the antibody gene research was new information about the possible causes of cancer, especially the blood cancers known as lymphomas and leukemias. These tumors originate in B lymphocytes and other immune cells and are often characterized by the presence of specific chromosomal abnormalities. In Burkitt's lymphoma, for example, the cancer cells are derived from B lymphocytes.

Lymphoma cells from about 90% of Burkitt's lymphoma patients have aberrant chromosomes formed by an exchange of segments between chromosomes 8 and 14. The gene for the antibody heavy chain is on chromosome 14, and investigators, including Leder, soon learned that chromosome 14 breaks in or near the site of the antibody gene during the exchange of chromosomal segments. Presumably, the DNA rearrangements that occur during the assembly of the heavy-chain gene increase the fragility of that region of the chromosome.

Moreover, the piece of chromosome 8 that becomes joined there carries the *myc* gene, one of several oncogenes that have been implicated in causing cancer. The supposition is that the normal control of the *myc* gene is lost when it moves, thereby contributing to the cancer development. The sites of the genes for the antibody light chains have also been linked to chromosomal abnormalities in Burkitt's lymphoma cells.

After spending 10 years in Basel, Tonegawa moved to MIT in 1981. In the years since, he has continued to explore the intricacies of the immune system. His contributions include the discovery of an "enhancer," an important gene control element, in the intron between the V-D-J and C coding regions of a heavy-chain gene.

In addition, Tonegawa's work has helped clarify the structure of the receptors with which T cells detect foreign antigens, an event that is necessary for activation of the cells. T cell activities include stimulating antibody production by B cells and killing virus-infected cells. The T cell receptor proteins are similar in structure to those of antibodies, and their genes are also assembled from three or four separate DNA segments.

Tonegawa has won a number of awards in addition to the Nobel. The most recent of these is the 1987 Lasker Award for Basic Medical Research, which he shared with Leder and Hood. Some observers expressed surprise that the Nobel was not also shared. But no one expressed surprise that Tonegawa's research was so honored. "His work was not just a single experiment," Waldmann says. "There was so much very nice work that this would be recognized." ■

JEAN L. MARX