ESSAY: AMERSHAM PHARMACIA BIOTECH & *SCIENCE* PRIZE

Transposition and Evolution of Antigen-Specific Immunity

Alka Agrawal

The vertebrate immune system is unique in its ability to mount an antigen-specific response to infection. It does so by producing large numbers of lymphoid cells, each expressing a unique antigen receptor: immunoglobulin in the case of B lymphocytes, and the T cell receptor in the case of T lymphocytes. The immune system is able to generate a vastly greater number of antigen receptors than would be possible if they were encoded individually in the germ line. Instead, antigen receptor genes are "split" into gene segments called V, J, and also D at some loci. Developing B and T cells use a highly intricate system of programmed DNA rearrangement called V(D)J recombination to assemble the variable portion of antigen receptor genes from these gene segments. This combinatorial system, combined with the fact that the gene segments are not joined together precisely, generates huge variability in the antigen recognition portion of the receptor (*1, 2*).

V(D)J recombination occurs only in developing lymphocytes and is absolutely dependent on the expression of the recombination activating genes *RAG1* and *RAG2* (*3, 4*). The reaction can be divided into two phases. The first phase involves recognition and cleavage of DNA at the border between a conserved recombination signal sequence and the flanking coding DNA segment. The second phase involves ligation of the cleaved recombination signals to form a signal joint, and addition and deletion of nucleotides to the coding DNA before ligation to form a coding joint. The first phase of the reaction is mediated by RAG1, RAG2, and the DNA bending proteins HMG1 or HMG2; the second phase of the reaction is mediated by ubiquitous proteins involved in the repair of DNA double-strand breaks (*5*).

To study the chemical steps of V(D)J recombination, our laboratory developed an in vitro system that performs regulated cleavage of a DNA substrate containing a pair of recombination signals (*6*). Using this system, I was able to demonstrate that after cleavage, a very stable complex forms between a pair of "synapsed" recombination signals containing RAG1, RAG2, HMG1 or HMG2, and components of the DNA-dependent protein kinase (*7*). The data provided an explanation for some in vivo observations about the kinetics of V(D)J recombination—namely, that although coding ends require a great deal of processing before being joined together, they are actually joined together quickly. In contrast, signal ends, which would appear to require only a simple ligation of two DNA ends, are joined together slowly, and apparently only after events that downregulate RAG expression (*8, 9*). We suggested that because the recombination signals are held together in this very stable complex after cleavage, additional steps must take place to remodel the complex and make the ends accessible to ligases. This would parallel events in bacteriophage Mu transposition (*10, 11*).

A long line of evidence had been accumulating suggesting that V(D)J recombination had evolved from a transposable element. The genomic organization of the *RAG1* and *RAG2* genes is very compact although the genes share no sequence similarity, the two genes are convergently transcribed, the cleavage mechanism is similar to transposition and retroviral integration, and the RAG proteins can join DNA together in a reversal of the cleavage reaction (*12–14*). This evidence led us to suspect that an unexpected low-mobility product of our in vitro cleavage reactions might be a circular intramolecular transposition product. This product would result from the RAG proteins cleaving DNA, then using the 3′ hydroxyls on the recombination signals to attack phosphodiester bonds in the DNA backbone. Upon sequencing the unexplained band, I found that this was exactly what had happened. The RAG proteins were not limited to catalyzing intramolecular transposition and could also catalyze intermolecular transposition into an exogenous piece of DNA, a defining feature of mobile elements. In both cases, the products contained a 3– to 5–base pair duplication flanking the recombination signals, confirming that the attacks were staggered, a characteristic feature of transposition and retroviral integration (*15*).

Amersham Pharmacia Biotech and *Science* are pleased to announce the 2000 grand prizewinner of the Amersham Pharmacia Biotech & *Science* Prize for Young Scientists. The grand prize has been awarded to a regional winner from North America, Alka Agrawal.

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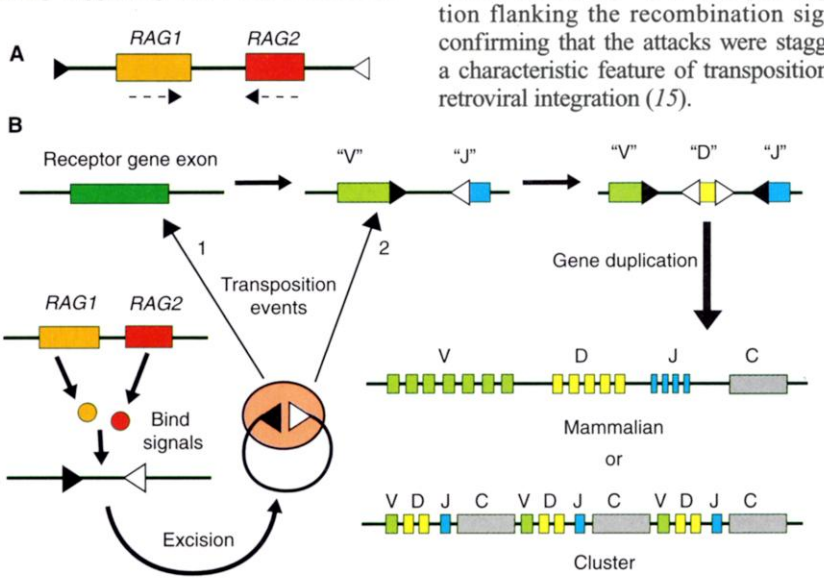

RAG-mediated transposition and a model for the origins of split antigen receptor genes. (**A**) Possible structure of the original transposable element that integrated into the germ line of an ancestral vertebrate. Dashed arrows indicate the direction of transcription of the *RAG1* and *RAG2* genes. (**B**) The current "split" nature of immunoglobulin and T cell receptor genes is proposed to have arisen from RAG-mediated transposition of one or two excised elements into a primordial receptor gene exon (dark green), thereby dividing the exon into two or three gene segments, each flanked by one or two recombination signals (black and white triangles). These gene segments would represent the evolutionary precursors of current V, D, and J gene segments. Different patterns of gene duplication (right) would result in the "mammalian" or "cluster" configurations of gene segments characteristic of the heavy-chain locus of mammals or cartilaginous fishes, respectively. The constant region exons (C) are represented as a single gray rectangle. [Adapted from (*15*)]

My data provide the strongest evidence to date that V(D)J recombination in fact evolved from a transposable element, with the RAG proteins constituting the transposase. Given that split antigen receptor genes are only found in jawed vertebrates, we proposed that these might have originated from a "RAG transposon" (see the figure). By analogy with other transposable elements, this would have consisted of recombination signals flanking the *RAG1* and *RAG2* genes. After insertion of the transposon into a receptor gene exon in the germ line of a jawed vertebrate ancestor, only excision of the element followed by DNA repair would allow the gene to be expressed (*12, 16*). Duplication of the gene segments could create the configuration of immunoglobulin and T cell receptor gene segments observed today.

The adaptive immune system arose suddenly in evolution around 450 million years ago, after the divergence of jawless and jawed vertebrates, coincident with the appearance of V(D)J recombination. The relatively sudden ability to produce antigen-specific lymphoid cells had long puzzled biologists and further bolstered the idea that a transposable element was involved. My work provided an elegant solution to this puzzle, and suggested that the fortuitous insertion of so-called "selfish DNA" led to a major weapon in the continual fight against pathogens.

This work begs the question: Does transposition still occur in vivo? This is still unclear, but there would be many reasons to suppress this activity. Continued transposition could be mutagenic and lead to leukemia by activating oncogenes, inactivating tumor suppressor genes, or creating chromosomal translocations. Errors in V(D)J recombination have long been suspected of causing leukemias, as many chromosomal translocations involve immunoglobulin or T cell receptor loci (*2*).

As the RAG proteins can catalyze a reaction identical to that catalyzed by retroviral integrases, the results also send a word of caution about designing antiviral drugs that target integrase, because these drugs could also inhibit V(D)J recombination during normal lymphocyte development.

References

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2000 Grand Prize Winner

Alka Agrawal grew up in Farmington Hills, near Detroit, Michigan, and earned her bachelor's degree in chemical engineering from the University of Michigan. Dr. Agrawal entered the pharmacology graduate school program at Yale University and began working in the laboratory of David Schatz, investigating the role of DNA repair proteins in V(D)J recombination. The development of an in vitro V(D)J cleavage system in the laboratory changed her focus and she began studying the cleavage mechanism as part of her doctoral research. Exposure to science policy at the National Academy of Sciences, and to science journalism through the AAAS Mass Media Science and Engineering Fellows Program, prompted Dr. Agrawal to pursue a career in science writing.

Alka Agrawal

Regional Winners

Europe: Rafal Ciosk for his essay "What Makes Sisters Inseparable" reporting his research on the mechanisms of sister chromatid cohesion and separation in budding yeast carried out in the laboratory of Dr. Kim Nasmyth at the Institute of Molecular Pathology in Vienna, Austria. Dr. Ciosk was born in Opole, Poland, and studied biology and genetics at the J.A.T.E. University in Szeged, Hungary. In 1995, he started his Ph.D. work in the laboratory of Dr. Kim Nasmyth and obtained his Ph.D. in genetics in January 1999. This year, he moved to Seattle, Washington, to study the germ cell precursors of *Caenorhabditis elegans* in the laboratory of Dr. James Priess at the Fred Hutchinson Cancer Research Center.

North America: Avraham Yaron for his essay "NFκB Activation: The Death of a Protein on the Way to a Transcript's Birth" reporting his Ph.D. research on the activation of immune system transcription factors, carried out in the laboratory of Dr. Yinon Ben-Neriah at the Lautenberg Center of Immunology at Hadassah Medical School in Israel. Dr. Yaron was born in Jerusalem and pursued undergraduate studies in the Hebrew University of Jerusalem from 1991 to 1993. He joined Dr. Ben-Neriah's laboratory in 1994 and set out to elucidate the activation pathway of the transcription factor, NFκB, and the part played by its inhibitor, IκB. In December 1999, he moved to the United States on an EMBO fellowship, joining the laboratory of Dr. Marc Tessier-Lavigne at the University of California, San Francisco, where he is now working on the signaling mechanisms of axon guidance receptors.

The second North American regional winner was Douglas Heithoff for his essay "When the Dam Breaks: Strategies of Bacterial Pathogens Exposed" based on his research in the laboratory of Michael J. Mahan at the University of California at Santa Barbara. Dr. Heithoff was born in Elmhurst, Illinois, and attended the University of Illinois at Urbana-Champaign where he received his bachelor's degree in biology in 1990. He joined the Mahan laboratory in 1993 where he identified *Salmonella* virulence factors required for host infection. Dr. Heithoff was awarded a Ph.D. in 1999 and is currently a postdoctoral research fellow in the Mahan laboratory.

Japan: Yuki Yamaguchi for his essay "Transcript Elongation—Stimulation Is Not Enough" based on his Ph.D. research carried out in the laboratory of Dr. Hiroshi Handa at Tokyo Institute of Technology (TIT). Dr. Yamaguchi was born in Chiba, Japan, and grew up in a suburb of Tokyo. He joined Dr. Handa's laboratory in 1994 as an undergraduate and studied various aspects of transcriptional regulation in higher eukaryotes. He then became interested in the regulation of RNA polymerase II during transcript elongation, and in his Ph.D. research identified a new class of proteins that directly control the polymerase's movement along the DNA. He received his Ph.D. from the Graduate School of Bioscience and Biotechnology, TIT, and is now a Research Fellow of the Japan Society for the Promotion of Science at TIT's Frontier Collaborative Research Center.

The full text of the essays written by the regional winners and information about applying for next year's awards can be seen on *Science* Online at www.sciencemag.org/feature/data/pharmacia/prize/apbprize.shl.