Similarly to how shuffling and dealing from the pack generates random hands of cards, the process of V(D)J recombination allows the random assortment of genes and the creation of highly variable antigen receptors. Credit: John McKenna / Alamy Stock Photo; modified by Nature Publishing Group.

MILESTONE 10

Building antibody diversity

With the central dogma well established by the 1960s, scientists were struggling to explain the observed diversity in antibodies: how are antibodies generated to the numerous, diverse antigens that organisms encounter? The debate was whether the diverse proteins are encoded by different genes (germline theory) versus whether the diversity results from variation that occurs during replication (somatic differentiation theory).

Accounting for drawbacks in both theories, Edelman and Gally proposed that few duplicated genes exist in tandem arrays with some point mutations, and that recombination occurs by somatic crossover during lymphocyte development, facilitated by the homologous regions and the proximity of these genes within arrays. The result would be two new sequences with different sets of point mutations. This breakthrough paper, published in 1967, was based on the observation that most substitutions occur at 'hot spots' in the variable region (V) of Bence-Jones proteins (dimers of light chain; MILSTONE 7), but it did not satisfactorily explain why the constant region (C) lacks such hypervariable segments.

Early experimental support for that model came in 1974 from a molecular biologist, Susumu Tonegawa, who was awarded a Nobel Prize for the work. He and his colleagues applied techniques he had used for studying viral transcription to the ongoing controversy over the origins of antibody diversity. A series of DNA-hybridization experiments with various κ-chain mRNAs revealed that there are not enough germline genes to explain observed diversity in the antibody repertoire. In 1976, a key paper by Hozumi and Tonegawa provided experimental evidence for the somatic rearrangement of immunoglobulin genes. They showed that V and C genes are well separated in the genome of mouse embryonic cells; however, when lymphocytes differentiate, the two sequences join to form a continuous V-C gene in both homologous chromosomes (a finding later confirmed by R-loop mapping).

Advances in sequencing added the joining region (J) into the picture, and the diversity region (D) was identified in the less-studied and harder-to-isolate immunoglobulin heavy chain. The next 5 years saw elucidation of the rearrangement process, which Frederick Alt and colleagues studied for the heavy-chain (H) locus in cells transformed by Abelson murine leukemia-virus. Notably, these cells had multiple distinct rearrangements close to J_H regions, but none near J_L regions, in most cases. In all cell lines, heavy-chain rearrangement started before light-chain rearrangement. Many reasons had been suggested for why the heavy chain is assembled first, and Alt and colleagues additionally proposed that adding light chains to a fully assembled heavy chain would help probe the success of light-chain gene rearrangement.

With V(D)J rearrangement established as the means of diversity generation, the recombination mechanism and enzymes had yet to be identified. In work from David Baltimore's laboratory, David Schatz and Marjorie Oettinger isolated recombination-activating gene 1 (RAG-1) and showed that genomic and cDNA sequences encoding RAG-1 activated V(D)J recombination in NIH-3T3 cells, albeit inefficiently. Furthermore, RAG-1 expression correlated with V(D)J recombinase activity, which led the group to propose that this protein activates recombination or is itself the recombinase. The latter is now known to be true, as RAG-1 works in concert with RAG-2 to make the DNA double-strand breaks that initiate V(D)J recombination.

Irene Kaganman,
Independent Editor

ORIGINAL RESEARCH PAPERS Alt, F. *et al.* Organization and reorganization of immunoglobulin genes in A-MuLV-transformed cells: rearrangement of heavy but not light chain genes. *Cell* **27**, 381–390 (1981) | Edelman, G. M. & Gally, J. A. Somatic recombination of duplicated genes: an hypothesis on the origin of antibody diversity. *Proc. Natl. Acad. Sci. USA* **57**, 353–358 (1967) | Hozumi, N. & Tonegawa, S. Evidence for somatic rearrangement of immunoglobulin genes coding for variable and constant regions. *Proc. Natl. Acad. Sci. USA* **73**, 3628–3632 (1976) | McBlane, J. F. *et al.* Cleavage at a V(D)J recombination signal requires only RAG1 and RAG2 proteins and occurs in two steps. *Cell* **83**, 387–395 (1995) | Oettinger, M.A. *et al.* RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. *Science* **248**, 1517–1523 (1990) | Schatz, D. G., Oettinger, M. A. & Baltimore, D. The V(D)J recombination activating gene, RAG-1. *Cell* **59**, 1035–1048 (1989) | Tonegawa, S. *et al.* Evidence for somatic generation of antibody diversity. *Proc. Natl. Acad. Sci. USA* **71**, 4027–4031 (1974)
FURTHER READING Schatz, D. G. & Ji, Y. Recombination centres and the orchestration of V(D)J recombination. *Nat. Rev. Immunol.* **11**, 251–263 (2011) | Schatz, D. G. & Swanson, P. C. V(D)J recombination: mechanisms of initiation. *Annu. Rev. Genet.* **45**, 167–202 (2011)