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Antibody Wars: Extreme Diversity

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People have pondered the Ab diversity problem for years as the universe of potential Ags was expanding relentlessly. Research showed that Abs could not only bind to pathogens such as bacteria, toxins, and viruses, but also to common proteins, like egg albumin, and synthetic chemical structures, like dinitrophenyl, that humans had never seen before. If the repertoire of Abs was unlimited, how was the genetic information for all these proteins encoded in the DNA? Two polar hypotheses emerged by 1970: the somatic theory, which states that a few inherited genes underwent high rates of somatic mutation to encode many Abs (championed by Weigert and Cohn; Ref. 1), and the germline theory, which states that many genes existed to encode each Ab (supported by Hood; Ref. 2). The somaticists argued that there was not enough DNA to encode all the Ab specificities, which most immunologists agreed was in the range of 10^6 unique molecules. The germliners countered that there was not enough time to generate a full repertoire somatically, and why do it randomly? Each side presented compelling data based on protein sequences, and the dialog was so fascinating that every immunologist had an opinion. The debate reached its zenith in the 1970s, when spin-off theories emerged: the translocation hypothesis, which states that V genes could recombine next to C genes (3); a complicated branched DNA model, in which RNA polymerase is shunted onto different DNA tracks (4); the gene interaction theory, where two sets of genes encode the framework and hypervariable regions of V genes (5); the permutation theory, which posits that germline genes undergo predetermined mutations (6); and the minigene hypothesis, where four sets of genes encode each of the four framework regions (7).

Buried in this plethora of theories was a short paper titled “Origin of Antibody Variation” by Brenner and Milstein (8), which described a hypermutation mechanism acting on a few genes. A simple figure accompanied this hypothesis, which showed a DNA motif located in the middle of a gene that is recognized by a cleaving enzyme, analogous to a restriction enzyme cutting at a site. The DNA is then degraded 3’ to 5’ by an exonuclease, and an error-prone polymerase fills in the gap with occasional errors. Thus, the left half is variable, and the right half is constant. The authors acknowledged that their error-prone repair model would be difficult to prove, but believed it was consistent with variable and constant regions.

Meanwhile, molecular biology was being unleashed in full force on Ab genes, and it was soon discovered that V and C genes indeed are separately encoded in the DNA. Even more amazing, the V gene was encoded by three separate gene segments—variable, diversity, and joining—that were represented by multiple members in the germline. Therefore, Ab diversity could be explained by combinatorial joining of gene segments within the three families. An added bonus was that random nucleotides were added during the joining process to further increase variability. Marrying both somatic and germline theories, gene segment joining should be enough to generate loads of diverse Abs, and everyone was satisfied.

However, it soon became apparent that B cells continued to mutate their Ab genes, even after rearrangement. Additional amino acid substitutions were found that were not encoded in the germline members and were not located near the sites of joining. These substitutions were completely random and tended to accumulate in hypervariable regions. Rearranged Ab genes from Ag-activated B cells then were sequenced extensively, and the data revealed an incredible number of mutations within the V gene and its flanking DNA. Both the high frequency and restricted targeting of these mutations were unprecedented in biology, and the somatic hypermutation theory was resurrected.

The Brenner and Milstein paper suddenly looked prescient. Molecular biologists validated the concept of DNA cleavage and gap formation in other repair processes, and the recent discovery of low-fidelity DNA polymerases would allow the synthesis of many mutations in a short region. In fact, the hypothesis that error-prone DNA repair causes Ab hypermutation is now dogma. In an interesting twist, Milstein originally developed hybridoma technology to study the hypermutation process in B cells. The creation of hybridomas secreting mAbs of choice was a huge success and earned him the Nobel Prize in 1984. Milstein went on to show that random mutations located in hypervariable regions are selected over time to produce high-affinity Abs (9). This is the fundamental basis of immunization and the raison d’être for hypermutation.

However, the story did not end until the molecular mechanism was solved. Going back to Fig. 1 in Ref. 8, what was the cleaving enzyme that started it? Discoveries by Tasuku Honjo and Michael Neuberger a few years ago unequivocally showed that a new protein, activation-induced deaminase (AID), initiates hypermutation by modifying Ig genes. AID is not a cleaving enzyme but acts on DNA by deaminating cytosine to uracil. Uracil is an inappropriate base and is recognized by error-prone DNA repair pathways to generate both DNA strand breaks and synthesize mutations, exactly as shown in the figure. AID also initiates H chain class switch recombination, which ties together V gene diversity with C gene diversity. In a poignant

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2 Abbreviation used in this paper: AID, activation-induced deaminase.
memory, Neuberger recalls that he showed Milstein, who was very ill, the draft figures for the first paper suggesting that AID deaminates cytosine in DNA. Milstein wanted to continue the discussion the following week that, for him, never came.

It is tempting to speculate about that early time when Francis Crick, Sydney Brenner, and César Milstein were together in the Laboratory of Molecular Biology in Cambridge. Apparently, Crick suggested that Brenner and Milstein collaborate and write a letter to *Nature* about their ideas on how diversity was generated. As Aaron Klug commented, the most remarkable fact is that they wrote the letter together at all since it was the sole example of a joint effort between them.

**Acknowledgments**

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**References**