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At the time of publication of a paper by Hozumi and Tonegawa (1) in 1976, a controversy was raging about the number of germline variable (V) genes and how Ab diversity was generated (2). Protein sequencing had clearly shown that Abs were divided into two contiguous regions: a V region and a constant (C) region. Dreyer and Bennett (3) had earlier proposed the two gene-one polypeptide theory, which implied that the genes would somehow be joined at the DNA or RNA level. A plethora of papers were swimming in the literature, which tried to estimate the number of V genes based on liquid hybridization of RNA to DNA, all of which gave inconclusive results because the RNA was not pure, etc. It was widely accepted that there were a handful of C genes, and probably many handfuls of V genes. If so, how would a V gene find its way next to a C gene?

Out of the blue, Hozumi and Tonegawa (1) published a stunning paper that used newly discovered restriction enzymes to digest DNA. They showed by hybridization that a V gene and a C gene are miles apart in germline DNA (from mouse embryos), but next to each other in Ab DNA (from a myeloma tumor). This was the first immunology experiment to use restriction enzymes and gel electrophoresis to deduce the position of genes in germline DNA and their repositioning during development. It is worthwhile to look at the techniques they used, because they are so different from the ones that were used just a few years later. First, they purified their own *Bam*HI enzyme. Nathans and Smith (Ref. 4; Nobel Prize 1978) had just described the use of restriction enzymes to analyze DNA, and there were only several enzymes available. Second, they poured a 2-liter agarose gel into a foot-long tray, cut the wells with a scalpel, and loaded 5 mg of DNA. This monster gel underwent electrophoresis for 3 days. Third, they cut the gel into ~30 slices to fractionate the DNA, put each slice into a tube, and heated them, and the DNA was then absorbed to hydroxyapatite and eluted. Southern blots were not widely used at that time. Fourth, each fraction was then annealed with ^{125}I-labeled mRNA prepared from a homogenous myeloma tumor. There were no radioactive nucleosides available, so the mRNA was chemically modified with ^{125}I to label cytosines. Nonannealed RNA was removed with RNase, and the resulting radioactivity in each fraction was plotted according to size. By the way, none of these experiments, including earlier protein sequencing, could have been done without the generosity of M. Potter (National Institutes of Health, Bethesda, MD) and his abundant collection of murine myeloma tumors. Hybridomas did not come along until a couple of years later by Milstein (Ref. 5; Nobel Prize 1984), which invaluably increased the repertoire of mAbs.

The results are convincingly displayed in a very simple figure (Fig. 2 of Ref. 1), which shows three peaks of radioactivity. One complexity was that there was no mRNA probe for V gene sequences, but there was a probe for the C gene, which was made by serendipitous nicking of the whole mRNA during iodination. Thus, the V sequences were determined indirectly from the difference in the two hybridization levels of the whole V + C molecule and the half molecule containing the C end. Fig. 2 of Ref. 1 showed two peaks corresponding to the V and C regions from embryonic DNA, which were not present in the myeloma DNA, and a third peak containing the joined V-C fragment in myeloma DNA, which was not present in the embryonic sample. Voilá! The V gene and the C gene are separated in germline DNA, and joined in differentiated DNA.

The authors correctly predicted the mechanism as occurring by either deletion or inversion, which is true for the κ locus they were studying. They incorrectly guessed that the absence of germline V and C peaks in the myeloma cells meant that one allele was lost, and the other was duplicated. In retrospect, they were fortunate to have chosen a myeloma that had similar V gene rearrangements, probably from the same family of V genes, on both alleles to simplify the pattern. It was also a good thing that they did not use the most common enzyme at that time, *Hin*dIII, because that would have cut in the intron between rearranged Vκ and Cκ, and the peaks would not have colocalized.

How did this paper affect what we know now? Gene rearrangements were unheard of in mammalian DNA — the immunologists knew they were onto something big. The field exploded in the next 15 years. Soon thereafter, the V gene was divided into three segments—variable, diversity, and joining, and all of these had to be rearranged, too. Studies of the mechanism of rearrangement led to discovery of heptamer-nonamer DNA sequences and finally to the elusive RAGs themselves. During ontogeny, it was found that the V genes were rearranged in linear order, and this led to mapping the genes along the chromosome and the chromatin accessibility model. The basis of allelic exclusion was elucidated. Plus, all these mechanisms then applied to rearrangement of the newly discovered TCR genes.

Susumu Tonegawa received the Nobel Prize in 1987 for his discovery of the genetic principle for generation of Ab diversity.

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