

The Genetics of Antibody Diversity¹

[Eileen Gregory](#) and [Jill Losee-Hoehlein](#)

Background: Antibodies are proteins made by mammals in response to foreign substances (antigens). These antibodies bind the antigen and target it for destruction by the immune system. One of the most important characteristics of antibodies are their specificity. An antibody is specific only for the antigen that stimulated its production.

Problem: At any one time the average human body contains antibodies that can react with 10^8 different antigens. This means that there are 10^8 different antibody proteins in the body. If each different protein is a result of a different gene, then it follows that there must be 10^8 different antibody genes. The problem is that according to the Human Genome Project the human genome contains around only 30,000 genes.

Solution: In 1976 Susumu Tonegawa proposed a mechanism of gene splicing to explain how the body can make so many different antibody molecules. In 1987 he received the Nobel Laureate in Medicine for discovering the genetic principle for the generation of antibody diversity.

Activity: Make sure that your students understand basic immunoglobulin structure, including the difference between the variable and constant regions, and light chains and heavy chains. Explain that the antigen binding region of the molecule is comprised of sections of both the light chain and the heavy chain.

1) DNA splicing of the light chain

The polypeptide chain that makes up the light chain is coded for by one variable (V) gene segment, one J (joining) gene segment, and one constant (C) gene segment. Give each student a ribbon of paper that contains the gene structure for the DNA coding for one type of light chain (the kappa chain). The ribbon should look like this:

V₁ V₂ V₃ V₄ V₅ / V₄₀ J₁ J₂ J₃ J₄ J₅ C

Explain that the one V gene segment is joined with one J gene segment which is attached to the constant region (C) gene segment. The bringing together of these gene segments is random and is done by DNA splicing. Since the constant part of the kappa light chain is always the same (thus the name constant), this gene segment only needs to appear once in the genome. Since the variable part of the light chain is what is different from antibody to antibody, there must be a variety of different variable (V) gene segments. The J or joining segment adds to the diversity. This DNA splicing mechanism allows the cell to produce a variety of different proteins, but uses little of the genome space since the C segment only needs to be present once, and the combination of a variety of V and J gene segments gives a multiplicity of segment combinations.

¹ Presented at the NSTA National Convention, Philadelphia, PA, March 27-30, 2003.

Have each student align one V gene segment with one J segment and paper clip them together so that the intervening DNA is looped out the back. This looped out DNA would be excised and permanently lost from the chromosome. Have them compare their DNA with another student's. *Would they make the same antibody light chain?*

Only the V and J segments that are adjoining would be expressed. Any other J gene segments between the splice site and the C gene segment would be removed later during RNA processing.

2) DNA splicing of the heavy chain

The polypeptide chain that makes up the heavy chain is coded for by one variable (V) gene segment, one diversity (D) gene segment, one J (joining) gene segment, and one constant (C) gene segment. Give each student a ribbon of paper that contains the gene structure for the DNA coding for the heavy chain. The ribbon should look like this:

V₁ V₂ V₃ V₄ V₅ / V₆₅ D₁ D₂ D₃ D₄ / D₂₇ J₁ J₂ J₃ J₄ J₅ J₆ C

Have each student splice the DNA for the heavy chain. The process is basically the same as for the light chains except that there are more V gene segments to choose from, and the final protein is made up of four gene segments (V, D, J and C) while the light chain is made up of only three (V, J and C). After they have aligned one V segment with one D segment with a paper clip, have them choose one J gene segment to join the V-D segment. Remind them that the intervening DNA that is looped out the back is spliced out and permanently lost to the cell. Have each student compare her/his heavy chain DNA with another student's. *Would they make the same antibody heavy chain?* Have the students compare their entire antibody molecule (light chain and heavy chain) with others in the class. *How many different antibody molecules have been made?*

3) Calculating antibody diversity

Give your students the following table to use in calculating the number of different antibody molecules made through gene splicing. *How many possible antibody molecules could be made through this splicing process?*

Gene Segment	Kappa Light Chain	Lambda Light Chain	Heavy Chain
Variable (V)	40	30	65
Diversity (D)	0	0	27
Joining (J)	5	4	6

Using this information the students should be able to calculate that:

- * There are 200 different kappa light chains (40×5) and 120 different lambda light chains (30×4).
- * Since an antibody can have either a kappa or lambda light chain there are 320 ($200 + 120$) different light chains.
- * There are 10,530 heavy chains ($65 \times 27 \times 6$).
- * Since the antigen binding site for each antibody is made up of one light chain and one heavy chain, there are 3,369,600 or 3×10^6 different antibodies due to random DNA splicing ($10,530 \times 320$).

Where does the remaining diversity come from?

When the gene segments are spliced together, additional nucleotides can be added to the splice site. Since the addition of nucleotides other than multiple of three will alter the reading frame of the rest of the polypeptide, this can greatly increase the protein diversity.

The antigen binding peptides of the variable region are also hypermutable regions; the DNA in these regions undergo a higher than average amount of mutation. This hypermutation also leads to increased antibody diversity.

Other exercises:

1. If the DNA splicing results in a non-productive rearrangement, the cell can continue to splice V, and J segments together. Have your students re-splice their light chain and heavy chain DNA to make a new antibodies. Remember that any DNA that had been looped out has been permanently lost to the cell. *How many new antibody molecules have been made in the class?*
2. The heavy chain C region is actually composed of several gene segments, each coding for one of the five antibody isotypes (IgM, IgD, IgG, IgE and IgA). The arrangement of the C gene segments is as follows:



IgM contains the C μ gene segment and is always the first antibody secreted by plasma cells. *Can you explain why?*

After the plasma cell has begun secreting IgM antibody, DNA splicing can occur so that other C gene segments are brought next to the VDJ gene segments. This is called isotype switching and will cause the plasma cell to begin to secrete a different type of antibody such as IgG, IgE or IgA. Again the DNA splicing results in the permanent loss of DNA between the two splice points. *Can a cell that is secreting IgA switch to secreting IgG?*

Resources: Immunology textbooks are great sources for more information on this mechanism. Two of my favorites are:

Immunobiology, The Immune System in Health and Disease, 5th Ed., C.A. Janeway, P. Travers, M. Walport, and M. Shlomchik. Garland Publishing. 2001.

Immunology, 5th Ed., R.A. Goldsby, T.J. Kindt, and B. A. Osborne. W. H. Freeman and Company. 2003.