Lecture 8: Introduction to Antibody Diversity.

B-Cell Development Overview (Checkpoints & DNA changes)

Four checkpoints:

- functional heavy chain
- functional light chain
- self-tolerance (Bone Marrow)
- self-tolerance (1st Lymph Node, *Follicular* dendritic cells)

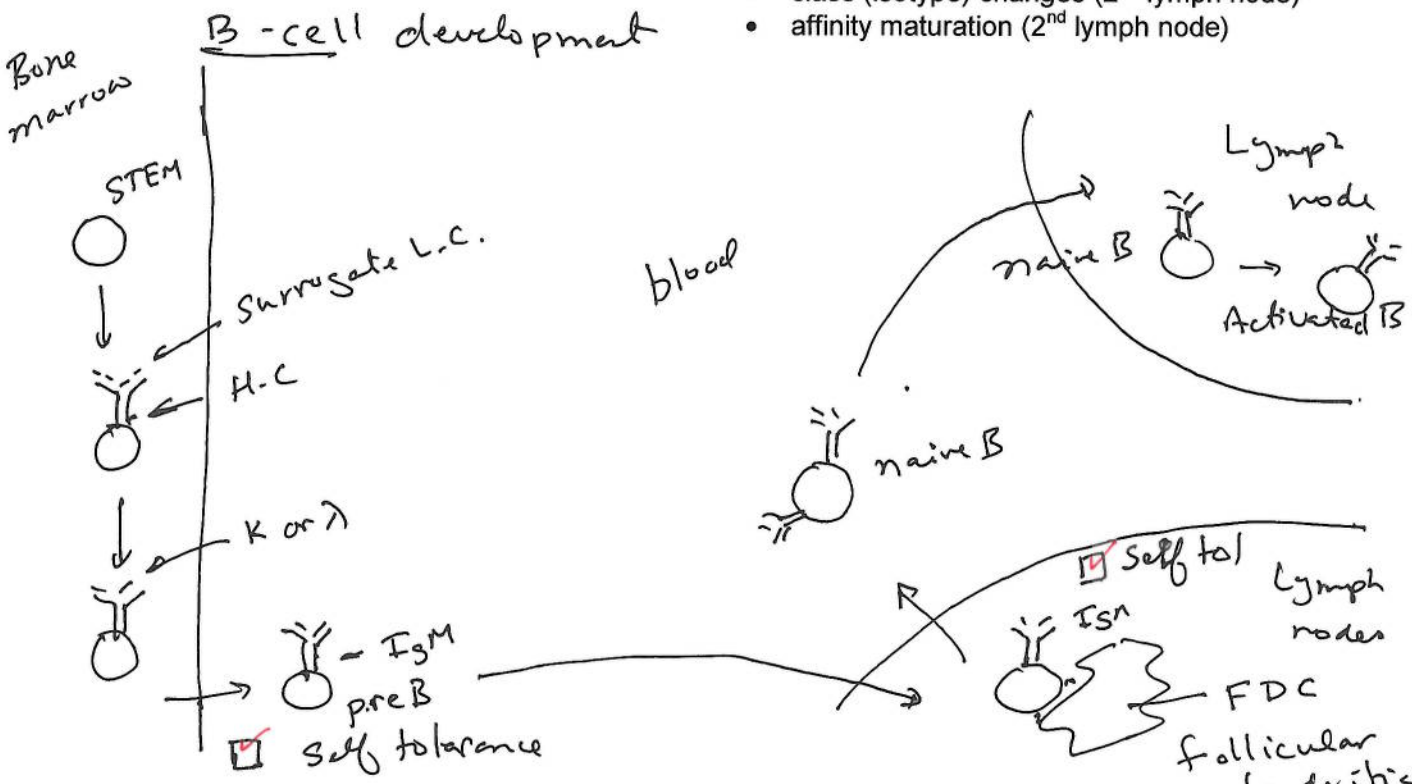
DNA changes:

In bone marrow:

- heavy chain generation
- light chain generation

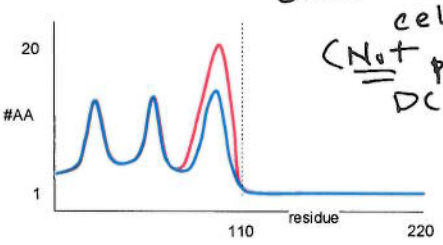
After activation:

- class (isotype) changes (2nd lymph node)
- affinity maturation (2nd lymph node)



Antibody Diversity: The following have to be accounted for:

1. Number of Different Specificities:
2. Constant region sequences:
3. Diversity thorough the variable regions: The amino acid sequences in the third hypervariable regions on both the heavy and light chains have been found to be more diverse than the first two hypervariable regions. The third hypervariable region on the heavy chain is more diverse than that on the light chain.



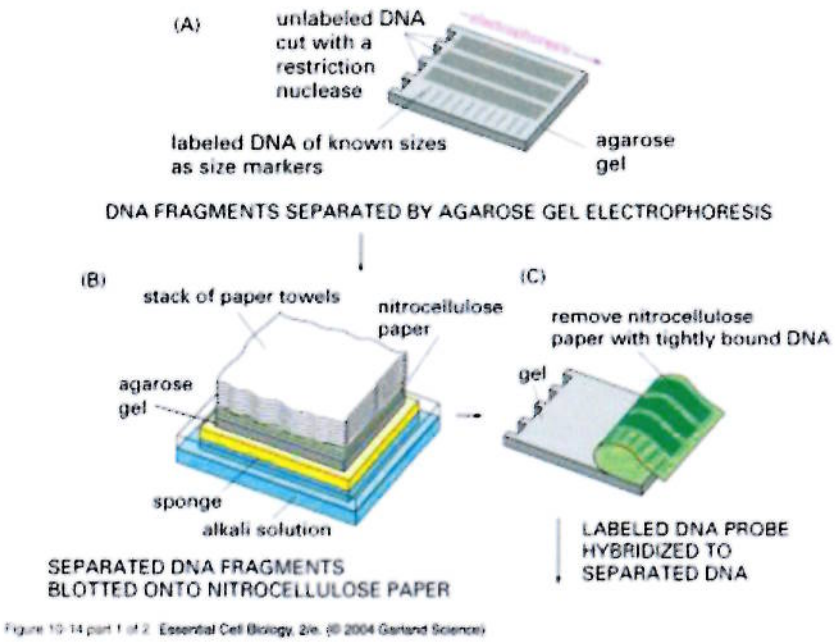
Historical models:

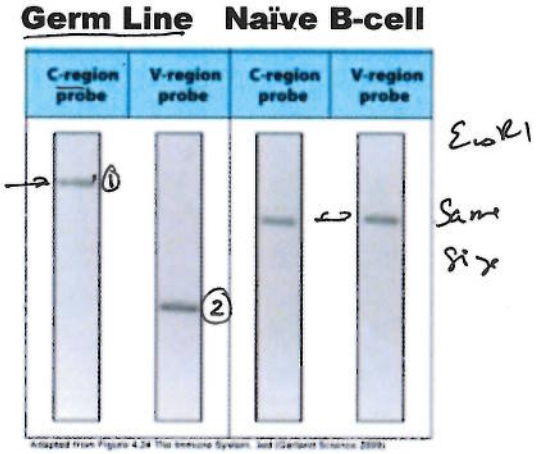
Germ line model:	*Somatic Mutation Model*
One gene = one protein, therefore many genes. How many? How much DNA is this?	One gene, mutates during development of B-cell. Problems with this model?

Tonagawa and Hozumi's Experiment: Compared DNA structure in stem and naïve B cells and detected changes in the size of DNA fragments after restriction digest of the DNA. Restriction enzymes cut DNA at specific sequences, e.g. EcoR1 cuts at GAATTC. Six base recognition sequences occur every 4Kb, on average.

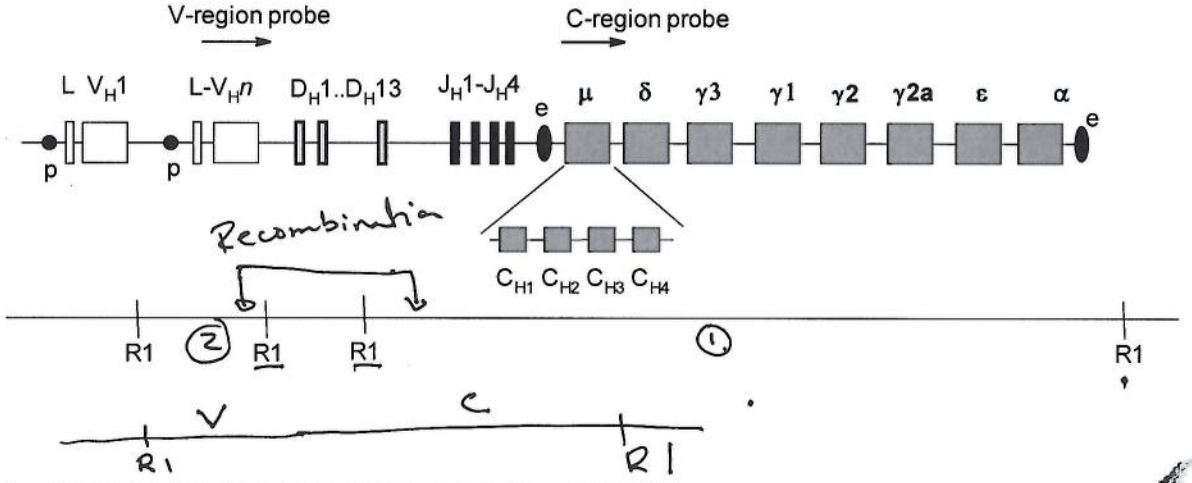
Southern blot technique:

i) Run DNA on agrose gel, separation by size.
ii) Transfer DNA to nitrocellulose filter, under denaturing conditions.
iii) Wash filter with radioactive labeled probe, probe binds to homologous DNA.
iv) Expose film, dark area indicates band containing homologous DNA.

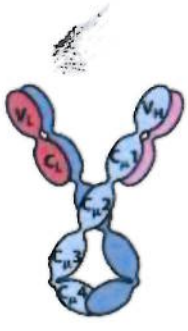




DNA sequencing produced the following structure of the heavy chain gene locus in mice **stem** cells:



The heavy chain locus contains the following segments



- Approximately ~300 separate "variable" regions, each with its own promoter (dot, p), leader peptide, indicated by 'L- and most of the variable region of the heavy chain, except for CDR3.
- Thirteen D domains that are found between the V segments and the J domain
- Four J domains found between the D and constant region sequences
- A series of segments that specify the different isotypes of the heavy chain appear to the 3' end of the J segments. These regions contain a number of transcriptional enhancers, "e".

What is the interpretation of Tonagawa and Hozumi's southern blots? What happens to the germ line DNA during B-cell Development?

The structure of the DNA changes → see lecture 9.