Theories for generation of Ab diversity:

Explain: diversity \((10^8)\), variable/constant regions/isotypes

- germ-line theory
- somatic-variation theory
- Dryer Bennet two-gene model
- verification of Dryer Bennet two-gene model

Multigene organization of Ig genes

- light chains: V, J and C
- heavy chain: V, D, J, C
In bone marrow:

1. Lymphoid cell
   - Expression of B220
     - Pro-B cell
       - Heavy-chain gene rearrangement
         - Immature B cell
           - Change in RNA processing
             - Mature B cell
               - Antigen stimulation
                 - Activated B cell
                   - Class switching and differentiation
                     - Plasma cells
                       - Mostly secreted Ig of various isotypes
                     - Memory B cells
                       - Ig expressed
                         - None
                         - \(\mu\) Heavy chain + surrogate light chain
                         - mIgM
                         - mIgM + mIgD
                         - Mostly mIg of various isotypes
Visualizing Concepts

(a) λ-chain DNA

L V_λ 1  J_λ 2  C_λ 2  J_λ 4  C_λ 4  L V_λ 1  J_λ 3  C_λ 3  J_λ 1  C_λ 1

5'  70 kb  1.2 kb  2.0 kb  1.3 kb  Pseudogene  19 kb  1.4 kb  1.7 kb  1.3 kb  3'

(b) κ-chain DNA

L V_κ 1  L V_κ 2  L V_κ n

5'  23 kb  2.5 kb  3'

n = 300

(c) Heavy-chain DNA

L V_H 1  L V_H n  D_H 1  D_H 13  J_H 1  J_H 4  C_μ  C_δ  C_γ 3  C_γ 1  C_γ 2b  C_γ 2a  C_ε  C_τ

5'  4.5 kb  55 kb  39 kb  21 kb  15 kb  14 kb  12 kb  3'

n = 300-1000
Variable-region gene rearrangements

- exon-intron structure

- site-specific DNA rearrangements only occur in lymphocytes

  single light-chain variable region; single heavy-chain variable region (immunocompetent)

  antigenically committed

Light-chain rearrangements:

\[ \lambda: V\lambda_1 \text{ can join with } J\lambda_1 \text{ or } J\lambda_3; \ V\lambda_2 \text{ can join with } J\lambda_2 \]

\[ \kappa: \text{ many more possibilities} \]

Heavy-chain rearrangements:

- 2 separate joining events
  DH to JH; DH-JH to VH to make VH-DH-JH (variable region)

- both IgM and IgD may be produced by the same B cell

Mechanism of gene rearrangements

Recombination signal sequences (RSS)—flank V, D, J gene segments

\[ \text{V-RSS}------\text{RSS-D-RSS}------\text{RSS-J} \]

RSS: conserved palindromic heptamer—12 or 23—nanamer seq.

\[ \text{V-RSS}------\text{RSS-D-RSS}------\text{RSS-J} \]

2-turn 1-turn 1-turn 2-turn

-one-turn/two-turn joining rule ensures correct recombination
(a) Nucleotide sequence of RSSs

CACAGTG 23 bp ACAAACAC
GTGTCAC 23 bp TGTTTTTGG

Heptamer Nonamer

Two-turn RSS

GGTTTTTGT 12 bp CACTGTG
CCAACACAC 12 bp GTGACAC

Nonamer Heptamer

One-turn RSS

(b) Location of RSSs in germ-line immunoglobulin DNA

λ-chain DNA

5' L V_λ ... J_λ C_λ 3'

κ-chain DNA

5' L V_κ ... J_κ C_κ 3'

Heavy-chain DNA

5' L V_H ... D_H J_H C_H 3'
Enzymatic joining of gene segments (VDJ recombinase)

- recombination occurs at junctions of RSSs and coding seq.
- recombination forms a coding joint and a signal joint
- transcriptional orientation of gene segments
  same $\rightarrow$ deletion; opposite $\rightarrow$ inversion
- recombination activating genes (RAG-1 and 2) lymphocyte specific
- terminal deoxynucleotidyl transferase (TdT)
- steps
- diversity in coding joints-CDR3 region
- identification of RAG genes
- defects in gene rearrangements
- productive and non-productive rearrangements
  joining flexibility $\rightarrow$ out-of-frame fusions
  other allele gets rearranged
- allelic exclusion
  rearranged genes expressed from only one chromosome
  expression of rearranged heavy and light-chain inhibit further rearrangement of the respective chains
- $\mu$ transgene expression (rearranged) in mice inhibits rearrangement in mice
(a) Deletional joining

1. Recognition of RSSs by RAG-1/2 and synapsis

2. Single-strand DNA cleavage by RAG-1/2

3. Hairpin formation and double-strand DNA break by RAG-1/2

4. Random cleavage of hairpin by endonuclease generating P-nucleotides

5. Optional addition to H-chain segments of N-nucleotides by TdT

6. Repair and ligation of coding and signal sequences to form joints by DSBR enzymes

(b) Inversional joining

1. Recognition of RSSs by RAG-1/2 and synapsis

2. Single-strand DNA cleavage by RAG-1/2

3. Hairpin formation and double-strand DNA break by RAG-1/2

4. Random cleavage of hairpin by endonuclease generating P-nucleotides

5. Optional addition to H-chain segments of N-nucleotides by TdT

6. Repair and ligation of coding and signal sequences to form joints by DSBR enzymes

△ = One-turn RSS
▽ = Two-turn RSS
Productive rearrangements:

1. Glu  Asp  Ala  Thr  Arg
   GAGGATGCGGACTAGG

2. Glu  Asp  Gly  Thr  Arg
   GAGGATGGGGACTAGG

3. Glu  Asp  Trp  Thr  Arg
   GAGGATTTGGGACTAGG

Nonproductive rearrangements:

4. Glu  Asp  Ala  Asp  Stop
   GAGGATGCGGGACTAGG

5. Glu  Val  Asp  Stop
   GAGGTGGGACTAGG
\[ \mu \text{ heavy chain inhibits rearrangement of } \mu \text{ allele } \#2 \text{ and induces } \kappa \text{ rearrangement} \]

\[ \mu + \kappa \text{ chains inhibit rearrangement of } \kappa \text{ allele } \#2 \text{ and } \lambda \text{ rearrangement} \]

Progenitor B cell

\[ D_H J_H \]

Productive allele \#1

\[ V_H D_H J_H \]

Productive allele \#2

Nonproductive allele \#1

Nonproductive allele \#2

Cell death

\[ \mu + \kappa \text{ chains inhibit } \lambda \text{ rearrangement} \]

\[ \mu + \lambda \text{ chains inhibit rearrangement of } \lambda \text{ allele } \#2 \]

Nonproductive allele \#1

Nonproductive allele \#2

Cell death
Generation of antibody diversity

1) multiple gene segments

2) combinatorial joining; heavy-chain=1.6 X 10^6

3) junctional flexibility of coding regions (out-of-frame too)

4) P-nucleotide addition
   - cleavage of hairpin by endonucleases
   
   short single-stranded region at end of coding sequence ↓

   addition of nucleotides by repair enzymes-palindromes (P)

5) N-region nucleotide addition
   - addition of nucleotides by TdT at coding joints
   - upto 15; CDR3

6) somatic hypermutation
   - rearranged variable regions can change further
   - million fold higher than normal mutation rate (substitutions)
   - Abs with highest affinity for Ag will get selected

   → affinity maturation

   - increased mutations after 2° and 3° exposure to Ag

7) association of heavy and light-chains
   - random associations alone = 1.9 X 10^9 combinations
   - other mechanisms included=10^{11}
<table>
<thead>
<tr>
<th>Pre-B cell lines</th>
<th>Coding joints (V_{k21} J_{k1})</th>
<th>Signal joints (RSS/RSS)</th>
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<tbody>
<tr>
<td>Cell line 1</td>
<td>5'-GGATCC GGACGTT-3'</td>
<td>5'-CACAGTG-3'</td>
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<tr>
<td>Cell line 2</td>
<td>5'-GGATC TGACGTT-3'</td>
<td>5'-CACAGTG-3'</td>
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<td>Cell line 3</td>
<td>5'-GGATCCGT GGACGTT-3'</td>
<td>5'-CACAGTG-3'</td>
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<tr>
<td>Cell line 4</td>
<td>5'-GGATCCGT GGACGTT-3'</td>
<td>5'-CACAGTG-3'</td>
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</table>

(a) P-nucleotide addition

**Hairpin**

\[
\begin{array}{ccc}
D & TC & 7 \\
A & AT & J \\
\end{array}
\]

Cleavage of hairpin 7 generates P-nucleotides

\[
\begin{array}{ccc}
D & TC & 7 \\
A & AT & J \\
\end{array}
\]

Repair enzymes add complementary nucleotides

\[
\begin{array}{ccc}
D & TC & 7 \\
A & AT & J \\
\end{array}
\]

(b) N-nucleotide addition

**Hairpin**

\[
\begin{array}{ccc}
D & TC & 7 \\
A & AT & J \\
\end{array}
\]

Cleavage of hairpin 7 generates P-nucleotides

\[
\begin{array}{ccc}
D & TC & 7 \\
A & AT & J \\
\end{array}
\]

TdT adds N-nucleotides

\[
\begin{array}{ccc}
D & TC & 7 \\
A & AT & J \\
\end{array}
\]

Repair enzymes add complementary nucleotides
<table>
<thead>
<tr>
<th>Hybridoma clone subclass</th>
<th>Heavy-chain V regions</th>
<th>Light-chain V regions</th>
<th>$K_d \times 10^{-7} M$</th>
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<td></td>
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<td>CDR2</td>
<td>CDR3 (D)</td>
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Class (isotype) switching

-- switch sites → located close to CH gene segments

-- TH cytokines induce switching to specific isotypes
e.g. IL-4 allows switching from Cμ to Cy1 or Cε

Expression of Ig genes

-- primary mRNA cleaved at polyadenylation site

-- examples of differential processing of mRNA

1) Expression of membrane or secreted Ig

   ↓

differential use of 2 polyadenylation sites

2) Expression of IgM and IgD

-- assembly of Igs

Regulation of Ig transcription

-- promoters/enhancers/silencers

-- effect of rearrangement on transcription

-- inhibition of Ig-expression on T cells

   3' κ-chain enhancer may be responsible for inhibition of Ig-expression in T cells
(a) H-chain primary transcript

5' \( \mu_1 \) \( \mu_2 \) \( \mu_3 \) \( \mu_4 \) S M1 M2 3' Poly-A site 1 Poly-A site 2

(b) Polyadenylation of primary transcript at site 2 \( \rightarrow \mu_m \)

5' L VDJ J S M1 M2 \( (A)_n \)

(c) Polyadenylation of primary transcript at site 4 \( \rightarrow \delta_m \)

5' L VDJ J \( \delta_1 \) \( \delta_2 \) \( \delta_3 \) S M1 M2 \( (A)_n \)