**A Prototype Ig Gene: Murine Kappa**

- About 100 $V_\kappa$ gene segments
- 4 J Gene Segment s
- 1 C$_\kappa$ Gene Segment

Multiple V gene segments, distant from J and C
A few J gene segments
One C gene segment

“GERMLINE” Ig genes are NOT transcribed or translated.

---

**IMMUNOGLOBULIN GENES UNDERGO TWO DNA REARRANGEMENTS**

- V(D)J Recombination: both light and heavy chains

Generates Diversity

- Class switch recombination: heavy chains only

Changes Isotype (antigen elimination)
V(D)J Recombination in the Kappa Locus

About 100
$V_\kappa$ gene segments

4 J Gene Segments

1 $C_\kappa$ Gene Segment

DNA is deleted

V(D)J Recombination in the Kappa Locus

V segments

$D_J$ rearrangement

V→DJ rearrangement
DNA Rearrangement Removes Sequences Between V, D and J Segments

RNA Splicing Removes Sequences Between J and C Segments

V(D)J recombination involves DELETION of DNA between V, D and J coding segments.

The deleted DNA is LOST from the cell because it is not replicated.
Recombination Signal Sequences (RSSs)
Flank Rearranging Gene Segments

RSS = heptamer, spacer and nonamer

| RSS=heptamer, spacer and nonamer | 7bp | 12/23 bp | 9bp |

### Figure 4-5

**A**

coding flank

CACAGTG  spacer  ACAAAAACC

12 or 23 bp

**B**

- IgH
- IgK
- IgA
- TCRα, TCRγ
- TCRβ, TCRδ

Figure 4-5 Immunobiology, 6/e (© Garland Science 2005)
RAG Proteins: Lymphocyte Specific and Uniquely Required for VDJ Recombination

RAG (Recombinase activating gene) 1 and 2 proteins INITIATE VDJ recombination:

1. Bind to the RSS sequences
2. Stabilize the synapse between two segments
3. Introduce a nick between coding region and RSS sequence; subsequent trans-esterification leads to hairpin structures on the coding sequences and blunt ends on the RSS sequences.
Components of general DNA repair FINISH
VDJ recombination:

Ku70/80
DNA-dependent protein kinase
Artemis
XRCC4
DNA ligase IV
Figure 4-7: Initiation of V(D)J rearrangement:
RAG-dependent cleavage

#1: Lymphocyte-Specific

- RAG protein complexes bind to 12 and 23 bp spaced recombination signal sequences (RSSs)
- The protein complexes bind to each other, bringing together the segments to be joined
- The DNA is cleaved to create hairpin structures at the ends of the immunoglobulin gene segments

#2: Constitutive

- Other proteins (Ku 70:Ku 80, and DNA-dependent protein kinases) bind to the hairpins and the cleaved RSS ends
- The DNA hairpins are cleaved at random. Additional bases may be added by terminal deoxynucleotidyl transferase (TdT) or subtracted by exonuclease to generate imprecise ends
- DNA ligase IV, along with XRCC4, joins the ends of the gene segments to form the coding joint and the RSS ends to

Resolution of cleavage products:
DNA non-homologous end-joining machinery

Figure 4-8: Addition of Bases at V-D-J Junctions:
An Important Source of Diversity

#“P” addition

- RAG complex binds to and cleaves recombination signal sequences to yield a DNA hairpin
- RAG-mediated cleavage of hairpin generates palindromic P-nucleotides
- N-nucleotide additions by TdT

#“N” addition

- Pairing of strands
- Unpaired nucleotides are removed by an exonuclease
- The gaps are filled by DNA synthesis and ligation to form coding joint
Mechanisms of junctional diversity (a)

A. Nucleotide deletions at junctions

Germline sequence:

$V_{\alpha} 21$: $\text{CCC} \text{CCC} \text{CCC} \text{TGG} \rightarrow J_{\alpha 1}$

Expressed sequence:

$\text{CCC} \text{CCC} \text{CCC} \text{TGG} \rightarrow \text{CCC} \text{CCC} \text{TGG}$

Out of frame sequence; not expressed:

$\text{CCC} \text{CCC} \text{CCC} \text{TGG} \rightarrow \text{CCC} \text{CTT} \text{GG}$

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 7-12a

Repeated rearrangements are possible at the light-chain loci

First VJ recombination

Nonproductive join

Second VJ recombination

Nonproductive join

Third VJ recombination

Figure 7-18 Immunobiology 6/e. (C) Garland Science 2005
CONSEQUENCES OF V(D)J RECOMBINATION

1. Combinatorial diversity: # of possible combinations is the product of the # of recombining segments i.e. for mouse h.c.: 120x20x4=10^4

2. Junctional diversity at CDR3
   - Deletion of bases at junctions
   - N region additions at junctions
   - P region additions at junctions

3. Allows receptor editing to alter potentially self-reactive antibodies

4. Activates transcription of the rearranged gene
   Juxtaposition of intronic enhancers with V region promoters.

Enhancer activates the promoter of the rearranged V gene, resulting in transcription.
### Ig HEAVY CHAIN LOCUS

1. **Membrane vs secreted exons**

2. **Mu and delta isotypes**

3. **Gamma, epsilon, and alpha isotypes**

**CLASS SWITCH RECOMBINATION (CSR)**
A second DNA rearrangement, unique to the HC locus

---

**Table:**

<table>
<thead>
<tr>
<th>Immunoglobulin</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
<th>IgM</th>
<th>IgA1</th>
<th>IgA2</th>
<th>IgD</th>
<th>IgE</th>
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</thead>
<tbody>
<tr>
<td>Heavy chain</td>
<td>γ1</td>
<td>γ2</td>
<td>γ3</td>
<td>γ4</td>
<td>μ</td>
<td>α1</td>
<td>α2</td>
<td>δ</td>
<td>ε</td>
</tr>
<tr>
<td>Molecular weight (kDa)</td>
<td>146</td>
<td>146</td>
<td>165</td>
<td>146</td>
<td>970</td>
<td>160</td>
<td>160</td>
<td>184</td>
<td>188</td>
</tr>
<tr>
<td>Serum level (mean adult mg ml⁻¹)</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>0.5</td>
<td>1.5</td>
<td>3.0</td>
<td>0.5</td>
<td>0.03</td>
<td>5 × 10⁻⁵</td>
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<tr>
<td>Half-life in serum (days)</td>
<td>21</td>
<td>20</td>
<td>7</td>
<td>21</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>2</td>
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</tbody>
</table>

**Figure 4-17** Immunology, 6/e, © Garland Science 2005
IgM and IgD Are Generated from a Single Primary Transcript by DIFFERENTIAL mRNA POLY A/SPLICING
mRNAs encoding both membrane and secreted forms of mu heavy chain are generated from a single primary transcript by differential splicing and polyadenylation.
“Germline” (I region) Transcripts Are Necessary For Isotype Switch Recombination

Cytokines regulate I region transcription:

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>IgM</th>
<th>IgG3</th>
<th>IgG1</th>
<th>IgG2b</th>
<th>IgG2a</th>
<th>IgE</th>
<th>IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>Inhibits</td>
<td>Inhibits</td>
<td>Induces</td>
<td></td>
<td>Inhibits</td>
<td>Induces</td>
<td></td>
</tr>
<tr>
<td>IL-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Augments production</td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Inhibits</td>
<td>Induces</td>
<td>Inhibits</td>
<td></td>
<td>Induces</td>
<td>Inhibits</td>
<td></td>
</tr>
<tr>
<td>TGF-β</td>
<td>Inhibits</td>
<td>Inhibits</td>
<td></td>
<td>Induces</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**AID** (Activation Induced Deaminase) is required for CSR

Deaminate ssDNA displaced by I transcript?

Initiates cleavage?

Subsequently, some repair proteins are involved: Ku70/80, XRCCR

---

**T cell secretes cytokines**

- Specific I region transcription
- Isotype switch recombination to specific $C_H$ gene segment

**TGFβ**

- IαSαCα RNA
- Cut and join $S_\mu$ and $S_\alpha$ DNA
- VDJCα mRNA
- IgA

---
<table>
<thead>
<tr>
<th>V(D)J Recombination</th>
<th>CSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Join in exon</td>
<td>Join in intron</td>
</tr>
<tr>
<td>RAGs required</td>
<td>RAGs Not required</td>
</tr>
<tr>
<td>Repair enzymes</td>
<td>Repair enzymes</td>
</tr>
<tr>
<td>Generates diversity</td>
<td>Changes isotype</td>
</tr>
<tr>
<td>Ag specificity</td>
<td>Ag elimination</td>
</tr>
<tr>
<td>Random</td>
<td>Regulated by T cell signals</td>
</tr>
</tbody>
</table>

1. Humans with mutations in gene products required for V(D)J recombination are immunodeficient:

   **RAG**  Various SCIDs, including Omenn’s syndrome

   **Artemis**  Radio-sensitive SCID

   **Ligase IV**  SCID with developmental deficiency

2. Humans with mutations affecting CSR have hyper-IgM AID mutations and other mutations