Ig Polypeptides Are Encoded by 
**Multiple Gene Segments**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>J</td>
</tr>
<tr>
<td></td>
<td>C</td>
</tr>
</tbody>
</table>

Light Chain POLYPEPTIDE

Light Chain GENE SEGMENTS

<table>
<thead>
<tr>
<th>Variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
</tr>
<tr>
<td>D</td>
</tr>
<tr>
<td>J</td>
</tr>
</tbody>
</table>

H.C. POLYPEPTIDE

<p>| |</p>
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{H1}</td>
</tr>
<tr>
<td>C\textsubscript{H2}</td>
</tr>
<tr>
<td>C\textsubscript{H3}</td>
</tr>
</tbody>
</table>

H.C. GENE SEGMENTS

**A Prototype Ig Gene: Murine Kappa**

About 10 \( V\kappa \) gene segments

4 J Gene Segments

1 C\( \kappa \) Gene Segment

Multiple V gene segments, distant from J and C

A few J gene segments

One C gene segment
Murine Ig Heavy Chain Gene Organization

~ 120 V Gene Segments ~20 Ds 4 Js 8 Constant Gene Segments

C_{\mu1} C_{\mu2} C_{\mu3} C_{\mu4}/S C_{\mu M}

L VH

Human Ig Loci

Figure 4-4: Immunobiology, 6/e, (c) Garland Science 2005
TCR Alpha and Beta Loci

TCR Delta and Gamma Loci
IMMUNOGLOBULIN GENES UNDERGO TWO DNA REARRANGEMENTS

1. V(D)J Recombination: both light and heavy chains
2. Class switch recombination: heavy chains only

DNA Rearrangement Removes Sequences Between V, D and J Segments

RNA Splicing Removes Sequences Between J and C Segments

Figure 4-2: Immunobiology, 4th ed. © Garland Science (2005)
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

<table>
<thead>
<tr>
<th>Chain</th>
<th>Heptamer</th>
<th>Spacing</th>
<th>Nonamer</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda )</td>
<td>CACAGTG</td>
<td>23</td>
<td>nonamer</td>
<td>GGTGGTGT CACTGTG</td>
</tr>
<tr>
<td>( \kappa )</td>
<td>GTGACAC</td>
<td>12/23 bp</td>
<td>nonamer</td>
<td>CAAAAACA GTGACAC</td>
</tr>
<tr>
<td>( H )</td>
<td>23</td>
<td>12</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4-5: Immunobiology, 6/e, (C) Garland Science 2005
Figure 4-7: Immunobiology of Lymphocytes (2nd Edition, 2000).

#1: Lymphocyte-Specific

Initiation of V(D)J rearrangement: RAG-dependent cleavage

- The protease 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.
- Other proteins (Ku 70/Ku 80, and DNA-dependent protein kinase) bind to the hairpins and the cleaved RSS ends.
- The DNA hairpins are cleaved at random, adding by terminal deoxynucleotidyl transferase (TdT) or substracted by exonuclease to generate imprecise ends.
- DNA ligases IV, along with XRCC4, joins the ends of the gene segments to form the coding joint and the RSS ends to coding joint.

#2: Constitutive

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

Figure 4-8: Immunobiology of Lymphocytes (2nd Edition, 2000).

An Important Source of Diversity

- 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.
- Pairing of strands.
- Unpaired nucleotides are removed by an exonuclease.
- The gaps are filled by DNA synthesis and ligation to form coding joint.
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.: $120 \times 20 \times 4 = 10^4$

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

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

4. Allows receptor editing to alter potentially self-reactive antibodies
Ig Polypeptides Are Encoded by Multiple Gene Segments

Variable Constant Light Chain POLYPEPTIDE

V J C Light Chain GENE SEGMENTS

Variable Constant H.C. POLYPEPTIDE

V D J C_{H1} C_{H2} C_{H3} H.C. GENE SEGMENTS

Heavy chain isotypes are generated by a second DNA rearrangement:

CLASS SWITCH RECOMBINATION (CSR)
<table>
<thead>
<tr>
<th></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><strong>Heavy chain</strong></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><strong>Molecular weight (kDa)</strong></td>
<td>146</td>
<td>146</td>
<td>165</td>
<td>166</td>
<td>970</td>
<td>160</td>
<td>160</td>
<td>184</td>
<td>188</td>
</tr>
<tr>
<td><strong>Serum level</strong></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>5×10⁻⁶</td>
</tr>
<tr>
<td><strong>Half-life in serum (days)</strong></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>
</tr>
<tr>
<td><strong>Classical pathway of complement activation</strong></td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Alternative pathway of complement activation</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Placental transfer</strong></td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Binding to macrophage and phagocyte Fc receptors</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>High-affinity binding to mast cells and basophils</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Reactivity with staphylococcal Protein A</strong></td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Figure 4-17 Immunobiology, 6/e (c) (c) Garland Science 2005)
**mRNA Splicing**

Mouse

```
J_H  C_p  C_3  C_γ3  C_γ1  C_γ2b  C_γ2a  C_ε  C_κ
```

Human

```
J_H  C_p  C_3  C_γ3  C_γ1  C_ε  C_κ1  C_κ2  C_κ4  C_κ  C_κ2
```

**DNA rearrangement: CSR**

**Figure 4-19** Immunobiology, 6/e, (© Garland Science 2005)

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**IgM and IgD Are Generated from a Single Primary Transcript by DIFFERENTIAL mRNA POLY A/SPLICING**

**Figure 4-20** Immunobiology, 6/e, (© Garland Science 2005)
mRNAs encoding both membrane and secreted forms of mu heavy chain are generated from a single primary transcript by differential splicing and polyadenylation.

CSR Involves DNA Deletion and Loss

Further rearrangement may occur
"Germline" (I region) Transcripts Are Necessary For Isotype Switch Recombination

Figure 9-7 © 2001 Garland Science

<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></td>
<td></td>
</tr>
<tr>
<td>IL-5</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Inhibits</td>
<td>Induces</td>
<td>Inhibits</td>
<td></td>
<td></td>
<td></td>
<td>Inhibits</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>

Figure 9-7 Immunobiology, 6th ed. © Garland Science 2005
T cell secretes cytokines

Specific I region transcription

Isotype switch recombination to specific $C_H$ gene segment

**V(D)J Recombination**
- Join in exon
- Repair enzymes
- Generates diversity
- Ag specificity
- Random

**CSR**
- Join in intron
- RAGs Not required
- Repair enzymes
- Changes isotype
- Ag elimination
- Regulated by T cell signals

TGF$_\beta$

IaS$\alpha$C$\alpha$RNA

Cut and join $S_\mu$ and $S_\alpha$ DNA

VDJC$\alpha$ mRNA

IgA
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

SUMMARY

1. Ig genes undergo two DNA rearrangements which result in loss of DNA: VDJ recombination and class switch recombination. TCR genes undergo VDJ recombination only.


3. VDJ recombination provides diversity thru recombinational mechanisms and junctional diversity; it also activates gene transcription.

4. CSR occurs in introns and requires AID (activation induced cytidine deaminase).

5. CSR allows changes in the heavy chain isotype, leading to different antigen elimination properties of the expressed antibody.

6. Defects in genes encoding RAG, AID and other factors cause human immune deficiency diseases.