A Prototype Ig Gene: Murine Kappa

About 100 V\textsubscript{K} gene segments

4 J Gene Segments

1 C\textsubscript{K} 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\textsubscript{K} gene segments

4 J Gene Segments

1 C\textsubscript{K} Gene Segment

DNA is deleted

V J C

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**

<table>
<thead>
<tr>
<th>RSS</th>
<th>7bp</th>
<th>12/23 bp</th>
<th>9bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_{H}</td>
<td>CAGCTGTG</td>
<td>23</td>
<td>CAAAACCC</td>
</tr>
<tr>
<td>V_{L}</td>
<td>GACGCAC</td>
<td>23</td>
<td>CAAAACCA</td>
</tr>
<tr>
<td>C</td>
<td>GTTTTGT</td>
<td>23</td>
<td>GTTACAC</td>
</tr>
</tbody>
</table>

**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

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

“N” addition

“P” addition

Figure 7-18
CONSEQUENCES OF V(D)J RECOMBINATION

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

2. Junctional diversity at CDR3
- Deletions 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

mRNA Splicing

DNA rearrangement: CSR

Expression of IgM

Expression of IgD

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:

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

- IgA mRNA

Cut and join S_M and S_α DNA

VDJCα mRNA

IgA
### V(D)J Recombination vs. CSR

<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>
</tbody>
</table>
| RAGs required       | RAGs \textbf{Not required}  
| AID is required     | |
| Repair enzymes      | Repair enzymes |
| Generates diversity | Changes isotype  
| Ag specificity      | Ag elimination |
| Random              | Regulated by T cell signals |

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