Lecture 13 – Segmental Joining & Diversity

Key Points:
- Joining specificity – RSS 1+2, 2+1
- Joining mechanism – coding joint
- P-nucleotides
- Exonuclease deletion
- N-nucleotides, TdT
- H&L pairing via C\textsubscript{\textgamma} & C\textsubscript{\textalpha1}

Segment Joining: During the recombination events that lead to the final form of the light and heavy chains there is a need to insure that the correct segments are joined. For example, you would not want to join two J segments together instead of a VJ joining event. The correct segments are joined because of two conserved recombination signal sequences (RSS) found directly adjacent to the V, J, and D regions in immunoglobin genes. One is called a one-turn and the other a two-turn RSS.

The position of RSS in the kappa light chain is as follows:

The position of the RSS in the heavy chain segment is as follows:

Pairing Rules for Correct Joining?

Joining Mechanism: The actual joining of DNA segments is catalyzed by two enzymes: RAG-1 and RAG-2 (Recombination Activating Genes). Steps are:
1. Alignment of the V/J junction via the RSS
2. Precise cleavage at the boundary of the coding and RSS on one strand
3. Formation of a hairpin structure at the ends of the V and J segments (see below)
4. Resolution of hairpin and joining of V and J, generating a coding junction.
5. Joining of the ends of the RSS, creating a signal joint. The circular DNA is lost from the cell.

| One-turn sequence | CACAGTG - 12 bp - AAAAAAAC
| Two-turn sequence | GTGTCAC - 12 bp - TGTGTTTG
|                  | GGTTCCTGGT- 23 bp - CACTGTG
|                  | CCAAAAACA- 23 bp - GTGACAC

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Hairpin formation: Cleavage at the RSS produces a hairpin structure at the end of each coding region due to attack of the 3'-OH on the top strand on the 5'-phosphate on the bottom strand.

Crossover Uncertainty (Junctional diversity) & P-nucleotides: The end points of the RSS are the nominal points for joining heavy and light chains. However, there is some uncertainty (up to 5 bases) where the rejoining of fragments occur. This leads to the loss/gain of codons, and subsequent amino acid changes in the third hypervariable loop. Frame shifts are also possible.

Cleavage on the top strand, followed by repair synthesis adds additional bases, generating palindromic sequences: P-nucleotides.

\[
\begin{align*}
5' & \text{TTACAGTG3'} \\
3' & \text{AATGTCAC5'} \\
\end{align*}
\]

\[
\begin{align*}
TTACAG & \rightarrow TTAC \\
AATGTC & \rightarrow AATGTCGA \\
\end{align*}
\]

Cleavage on the lower strand, followed by exonuclease digestion leads to the loss of bases:

\[
\begin{align*}
--V----&\rightarrow-1-- \\
5' & \text{TTACAGTG3'} \\
3' & \text{AATGTCAC5'} \\
\end{align*}
\]

\[
\begin{align*}
TTACAG & \rightarrow TTA \\
AATGTC & \rightarrow AAT \\
\end{align*}
\]

Example of Crossover Uncertainty: Sequences A, B, or C are possible sequences after rearrangement of germ line to generate light chains (germ line sequence is shown in middle).

\[
\begin{align*}
A & \text{AGT CGC TTA CCT GCT GCT TTT} \\
B & \text{AGT CGC TTA CCT GCT TTT} \\
C & \text{Ser Arg Leu Pro Ala Ala Phe} \\
D & \text{Ser Arg Leu Pro Ala Phe} \\
\end{align*}
\]

V\textsubscript{3}-region (germ line) \textsubscript{J}\textsubscript{1}-region

\[
\begin{align*}
\text{AGT CGC TTA CCT} & \text{-----2GCT GCT TTT} \\
\text{TCA GCG AAT GGA} & \text{-----2CGA CGA AAA} \\
\text{Ser Arg Leu Pro} & \text{-----Ala Ala Phe} \\
\end{align*}
\]

N-Nucleotides: Addition of up to 15 nucleotides, by the enzyme terminal transferase (TdT). This enzyme adds bases to hypervariable loop 3 of only the heavy chain at each joining event. The added nucleotides are referred to as N nucleotides. The expression of TdT is very low when the light-chain begins rearrangement; consequently N-nucleotides are rare in the light chain.

Summary of Diversity:

<table>
<thead>
<tr>
<th>Mechanism of Diversity</th>
<th>Heavy Chain</th>
<th>Light Chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combinatorial V-D-J and V-J:</td>
<td>300 × 12 × 4 = 1.4 × 10^4</td>
<td>300 × 4 = 1.2 × 10^3</td>
</tr>
<tr>
<td>P base (V-D-J) (\times 3) (joint)</td>
<td>× 9</td>
<td>× 3</td>
</tr>
<tr>
<td>Junctional Diversity (\times 3) (joint)</td>
<td>× 9 (VDJ)</td>
<td>× 3 (VJ)</td>
</tr>
<tr>
<td>N-base addition (TdT) (V-D-J)</td>
<td>× 9</td>
<td>× 1</td>
</tr>
<tr>
<td># Chains</td>
<td>~1.0 × 10^7</td>
<td>~1.0 × 10^4</td>
</tr>
<tr>
<td>Estimated Diversity</td>
<td>1.0 × 10^11</td>
<td></td>
</tr>
</tbody>
</table>