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Antigen Receptors and The Generation of Diversity

Recommended reading: Abbas et al., 6th edition, Chapters 6 and 7
Janeway et al., 5th edition, Chapters 3 and 4)

The antigen receptors on B and T lymphocytes (the B Cell Receptor or BCR, and the T Cell Receptor or TCR) are broadly similar in structure and are believed to initiate signaling in similar ways. The antigen binding chains of these receptors do not directly contact cytosolic signaling molecules, but interact with accessory proteins which are also anchored in the plasma membrane and whose cytoplasmic tails contain motifs known as ITAMs (Immunoreceptor Tyrosine based Activation Motifs). ITAMs contain the following consensus sequence:
$YxxL/Ix_{6-8}YxxL/I$

These motifs are found in proteins associated with a number of signaling receptors in the immune system. When tyrosine residues in "YxxL/I" tetrapeptides are phosphorylated, they may be recognized by specific SH2 domains (present in certain proteins. SH2 domains or Src homology 2 domains are defined based on their homology to an approximately 100 amino acid domain originally described in the Src gene that encodes a very well studied non-receptor tyrosine kinase.SH2 domains each recognize specific phosphotyrosine containing peptide motifs. Phosphorylated ITAMs are therefore capable of recruiting molecules that contain specific SH2 domains to activated antigen receptors. Another domain commonly found in many signaling molecules is an SH3 (Src homology 3) domain also originally described in the Src kinase. SH3 domains are also about a 100 amino acids in length and associate specifically with proline rich stretches in proteins.

The TCR is made up of either α and β chains (in αβ T cells) or γ and δ chains (in γδ T cells). These antigen binding transmembrane polypeptides are linked to one another by a disulfide bridge. Each heterodimer is associated with the CD3 complex made up of integral membrane proteins which contain ITAMs in their cytoplasmic tails. The antigen receptor on B cells is made up of membrane immunoglobulins (IgM and IgD in naive B cells; IgG, IgA, or IgE in some activated and memory B cells) associated with a disulfide linked heterodimer made up of two integral membrane glycoproteins, Igα and Igβ. These latter proteins each contain an ITAM in their cytoplasmic tails. The cytoplasmic tails of the μ and δ heavy chains contain only three amino acids (KVK) which basically function as a "stop transfer" sequence during the process of translocation into the ER and do not present a significant surface for the association of these receptors with cytosolic signal transducers. The ITAMs in Igα and Igβ are critical for signal transduction) by the B cell receptor.

Although antigen receptors may potentially initiate signals in multiple ways, the major mechanism by which signals are generated is the activation of tyrosine phosphorylation. Src-family kinases associate with the antigen receptor even in quiescent

cells. The cytoplasmic tails of co-receptor proteins (proteins on lymphocytes which associate with the antigen recognized separately by the antigen receptor) also bind to Src-family kinases.
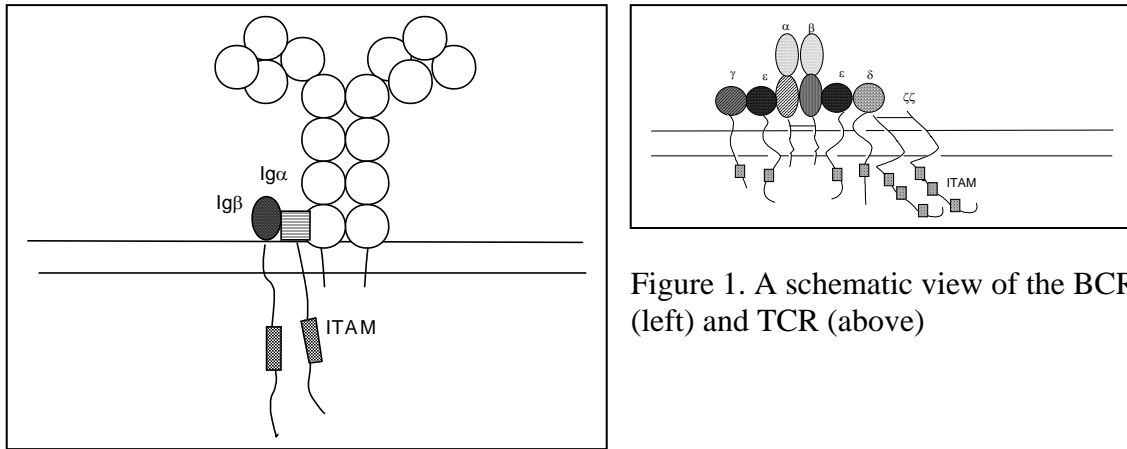

Figure 1. A schematic view of the BCR (left) and TCR (above)

The generation of diversity

The tremendous diversity of the immune system is largely generated in developing B and T cells by the process of gene rearrangement. This process is intrinsically designed to be somewhat inaccurate - the addition or removal of bases during the joining process contributes to the generation of diversity. As a result rearrangement is intimately tied to selection processes. Cells that make productive rearrangements are selected to survive and differentiate further while cells that fail to do so die by default. The rearrangement of immunoglobulin and T cell receptor genes is initially activated in pre-B and pre-T cells and because it involves the joining of V (variable) region genes to D (diversity) segments, and of D segments to J (junctional) regions, this process is known as **V(D)J recombination**. At the pre-B stage VDJ recombination generates rearranged heavy and light chain loci. At the immature B stage, cells that encounter multivalent self-antigen are given an opportunity to reform themselves or to die. Signals delivered by the B cell receptor reactivate the process of V(D)J recombination; cells that successfully make a new receptor by a process that is known as **"receptor editing"** may evade death and become useful citizens.

In the case of antibody genes, the heavy chain locus and the light chain loci are on different chromosomes. In the heavy chain locus a large number of V (variable region) genes are tandemly arranged upstream of about 20-30 D (diversity) segments. A few J segments are separated by the J-C intron from heavy chain constant region (C) exons. Both combinatorial and junctional mechanisms contribute to diversity. D segments are found in the Ig heavy chain locus and the TCRβ and δ loci. Two VDJ recombinational events are involved in the joining process at these loci. D segments are lacking in the Ig light chain, TCRα and TCR γ loci.

Rearrangement is an ordered process. The first rearrangement that occurs in a cell committed to the B lineage, for instance, is a D-J rearrangement at the heavy chain locus. This event is followed by V to DJ rearrangement and sequential rearrangement at the κ and λ light chain loci.

All immunoglobulin and T cell receptor gene segments which undergo rearrangement are flanked by recombination signal sequences (RSSs). Immediately

adjacent to the coding region is a conserved heptamer (CACTGTG or its reverse complement CACAGTG) followed by a spacer and then a relatively conserved nonamer (GGTTTTTGT or its reverse complement ACAAAAACC).

The spacer in an RSS is either approximately 12 bp in length or approximately 23 bp in length. RSSs witth 12 bp spacers recombine only with RSSs with 23 bp spacers (**the 12/23 spacer rule**).

The entire process of VDJ recombination can be divided into three steps:
1. Cleavage of the DNA to generate a double stranded break
2. Processing of the cut ends - primarily to generate greater diversity
3. Joining the processed ends.

However the division of the process into these three steps is somewhat artificial. Molecules such as Rag-1 and Rag-2 which are involved in the cutting step are also probably required for joining.

The RAG-1 and RAG-2 proteins recognize RSSs and specifically cut the DNA in a flush end between the heptamer and the coding sequences.RAG-1 and RAG-2 may form a dimer and are both needed for recombination. These proteins are nuclear proteins; little is understood about the structural basis for the function of Rag-1 and Rag-2.

RAG-1 binds to nonamer sequences in RSSs and probably recruits RAG-2. It is possible that a tetrameric RAG complex is formed. The RAG-1 /RAG-2 complex may bring together RSSs containing 12 bp and 23 bp spacers and probably only when this has been achieved, initiate the cutting process. At the heptamer-coding sequence junction, RAG-1/RAG-2 first makes a nick and the free nicked end attacks the other strand generating a closed hairpin structure at the coding end. A flush heptamer signal end results from the double-stranded break.

Blunt signal ends are joined without any processing. However the hairpin coding ends are opened up and nucleotides may be added to or removed from these ends resulting in greater immune diversity (and a fair number of out-of frame rearrangements).

Template-dependent and template-independent mechanisms exist for the addition of nucleotides to coding ends. Terminal deoxynucleotidyl transferase (**TdT**) is an enzyme responsible **for N region** addition. N regions are short stretches of bases, usually GC rich, that are found at junctions and which are added in a template independent manner. Template dependent nucleotide addition occurs when hairpins are cleaved eccentrically resulting in a few additional nucleotides in a single stranded extension. These extra nucleotides are retained in coding joints and are called P nucleotides. An endonuclease called Artemis plays a critical role in opening up the hairpin. Artemis mutations are a cause of SCID (severe combined immunodeficiency)
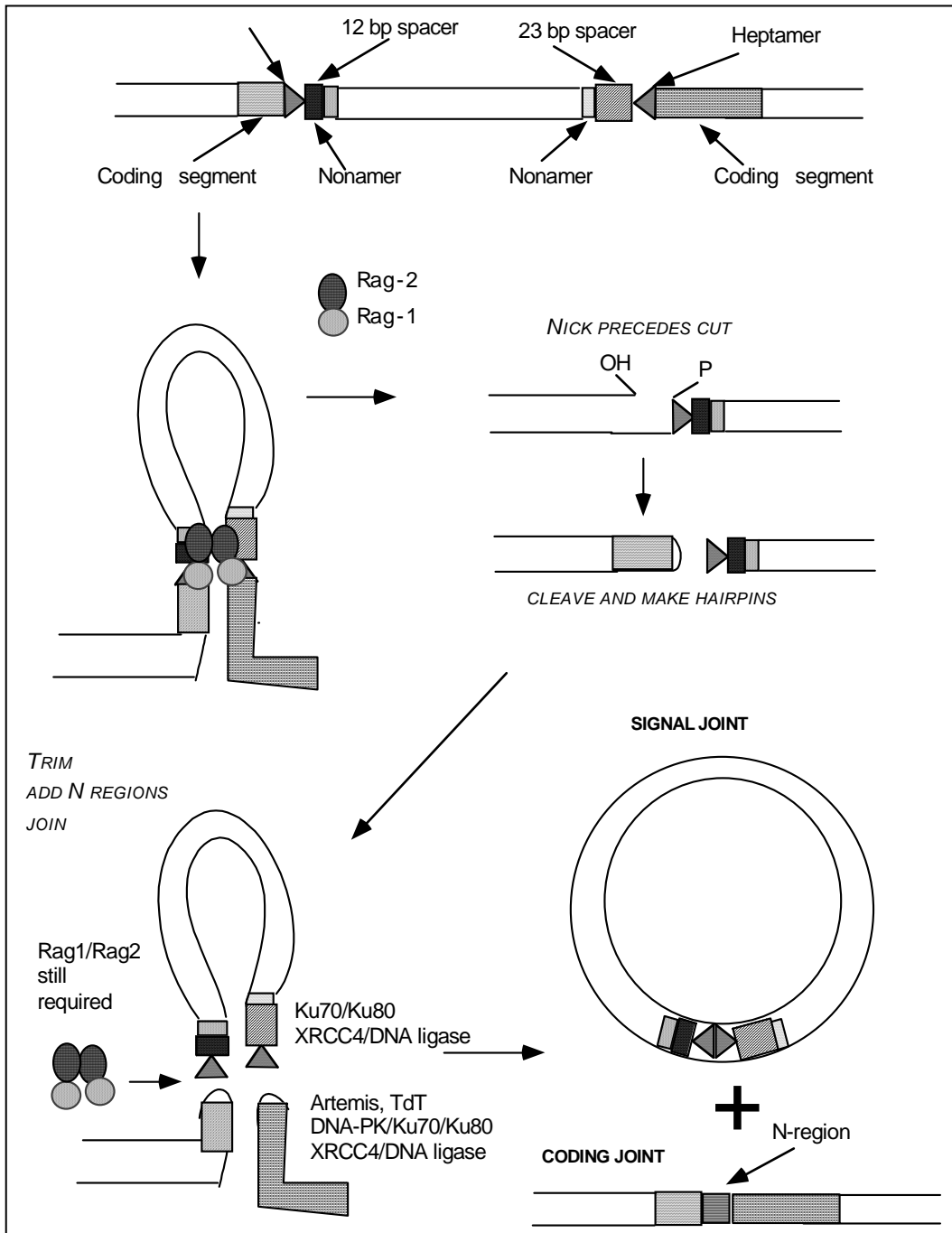
Figure 2. A schematic view of the steps involved in VDJ recombination.

Proteins that are involved in Double Strand Break Repair (DSBR, also known as Non-Homologous End Joining or NHEJ) are critical for the joining process in VDJ recombination. These genes include Ku70, Ku 80, DNA dependent protein kinase (DNA-PK), XRCC4 and DNA ligase IV. DNA-PK is the *scid* gene product and forms a complex with Ku70 and Ku80. The scid gene is so named because it is defective in murine Severe Combined Immune Deficiency. In the absence of DNA-PK, Ig and T

cell receptor rearrangement is extremely inefficient and scid mice basically lack B and T cells. DNA-PK is a member of a growing family of dual lipid/protein kinases, prototypic members including PI-3 kinase and the ATM (ataxia telangiectasia mutated) protein. The Ku 70 and Ku 80 proteins are non-catalytic subunits of DNA-PK, and are capable of binding broken DNA ends and aligning them for repair The most important substrate of DNA-PK during DSBR is probably the histone H2A variant called H2AX. Phosphorylated H2AX is probably required for retaining reair proteins at the site of a break.

Table: Selected DSBR proteins and their functions during NHEJ

Ku80/Ku86	Binding and aligning broken ends; non catalytic subunits of DNA-PK
DNA-PKcs	Phosphorylation of a number of substrates, most critical being histone H2AX
XRCC4	Subunit of DNA Ligase IV complex
DNA Ligase IV	Covalently joining broken ends

It is important that a given lymphocyte express only a single receptor in order to maintain clonal specificity. During B and T cell development pro-B and pro-T cells that make in-frame rearrangements of the IgH or TCRβ chain genes respectively, synthesize pre-antigen receptors. These pre-antigen receptors provide survival and proliferative signals and shut off rearrangement at these loci. These signals mediate **allelic exclusion** by making sure that only one allele of a receptor chain is productively rearranged. A number of human immunodeficiency disorders results from defects in V(D)J recombination or pre-antigen receptor mediated pre-B or pre-T cell survival. Mutations in Rag genes are found in some forms of Severe Combined Immunodeficiency (SCID) and mutations in components of the pre-B receptor and signaling components downstream of this receptor result in serious humoral immunodeficiencies.

A brief list of the mechanisms involved in combinatorial and junctional diversity is provided below:

Combinatorial Diversity:
1. Pairing of of Ds and Js at H chain locus
2. Pairing of Vs at H-chain locus with rearranged DJ
3. Pairing of Vs with Js at light chain locus
4. pairing of each rearranged H-chainwith different L-chains

Junctional Diversity
1. N regions
2. P nucleotide addition

Summary:

■ The BCR and TCR contain antigen binding chains and associated transmembrane proteins that contain ITAMs (Immunoreceptor Tyrosine-based Activation Motifs. Src-family kinases are loosely associated with these receptors.

- The TCR is composed of two transmembrane chains (either αβ or γδ) linked by a disulfide bridge and associates with the CD3 complex.

- The BCR consists of membrane-bound Ig's. The BCR associates with an ITAM-containing heterodimer (Igα and Igβ).

- Tyrosine kinases involved downstream of antigen receptors and Src-family kinases include Syk and the related Zap 70 kinase and Tec kinases (such as Btk, Itk and Rlk).

- Diverse Ig and TCR genes are created by recombination of antigen receptor gene segments. Conserved DNA sequences known as recombination signal sequences (RSS's) flank each V (variable), D (diversity), and J (junctional) segment of the TCR and BCR gene loci and are recognized by the RAG enzymes for recombination during lymphocyte development. Each RSS consists of a heptamer and a nonamer sequence separated by either a 12 bp or 23 bp segment. The 12/23 spacer rule says that a 12 bp spacer can only recombine with a 23 bp spacer (thus, preventing V-V recombination, for instance).

- There is a temporal order to the rearrangement process. Heavy chain gene rearrangement precedes light chain gene rearrangement Note that the D segments are only found in the heavy chain locus of the BCR and in the TCRβ and δ loci. For IgH and TCR loci D to J recombination occurs prior to V to DJ. joining

- During recombination, the RAG enzymes are involved in cleavage of the DNA to generate a double strand break which forms hairpins in the coding DNA.

- Combinatorial diversity is generated by the random joining of different V, D, and J segments and the pairing of different rearranged heavy and light chains;

- Junctional diversity is obtained by uneven cleavage of the hairpins resulting in the addition of P nucleotides to fill in the single-stranded portion; and N nucleotides which are added to the ends of the cleaved DNA in a template-independent manner by the enzyme TdT.

- Proteins involved in joining broken DNA ends include DNA-PK (mutated in SCID), and its associated subunits Ku70, and Ku80, and also DNA ligase IV and its partner XRCC4

■ Mutations in the genes essential for V(D)J recombination result in human immunodeficiency disorders.

Objectives/ StudyQuestions

1. What function is mediated by an ITAM motif?
2. What are coreceptors? How are they different from costimulatory receptors?
3. What is the 12/23 rule?
4. Name the lymphoid-specific factors involved in V(D)J recombination. What do they do?
5. What are N regions? What type of diversity do they contribute to?
6. What is the order of rearrangement of Ig genes and gene segments in the B lineage?
7. Which chain of the T cell receptor is "developmentally homologous" to the Ig heavy chain?
8. What ubiquitous proteins are required to complete the process of V(D)J recombination?