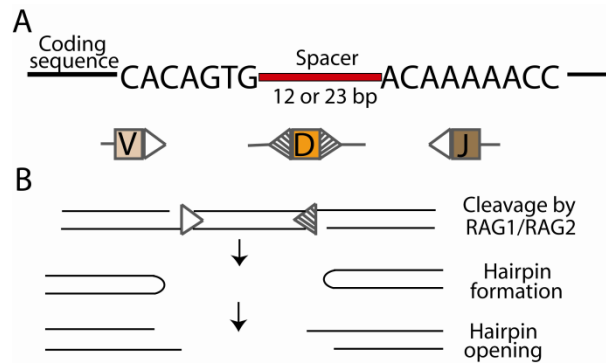## V-D-J Recombination (mechanism and regulation of V-D-J rearrangement)


Target sequences for the RAG1/RAG2 recombinase

The RAG1/RAG2 target sequences are called RSS, or recombination signal sequences. They flank the coding regions for the V, D and J segments and mark the site for DNA breakage and joining. An RSS consists of a conserved heptamer and a nonamer separated by a 12 bp or 23 bp spacer. Recombination occurs efficiently between an RSS(12) and an RSS(23) but not between two RSS(12) or RSS(23) units. The arrangement of the RSS(12) and RSS(23) at the V, D and J segments is such that V to D joining and D to J joining can occur efficiently but V to V joining, D to D joining and J to J joining are quite unlikely. In the diagram, RSS(23) is denoted by the open triangle, RSS(12) by the shaded triangle. Recombination is promoted between an open and a shaded triangle.

Recombination by RAG1/RAG2 is mechanistically related to DNA transposition

The RAG1/RAG2 DNA cleavage generates a 3'OH group at the coding sequence ends. These hydroxyls attack the phosphodiester bonds on the opposite strands to generate hairpins at the coding ends (the reaction is mechanistically similar to what happens during the excision of the Tn10 transposon). The hairpin ends are opened by hydrolysis (water attack) to generate free DNA ends. The V end will then be joined by DNA ligase to the D end (or the D end to the J end) to generate a V(D)J joined DNA region.

As we discussed in class, the joining of the coding
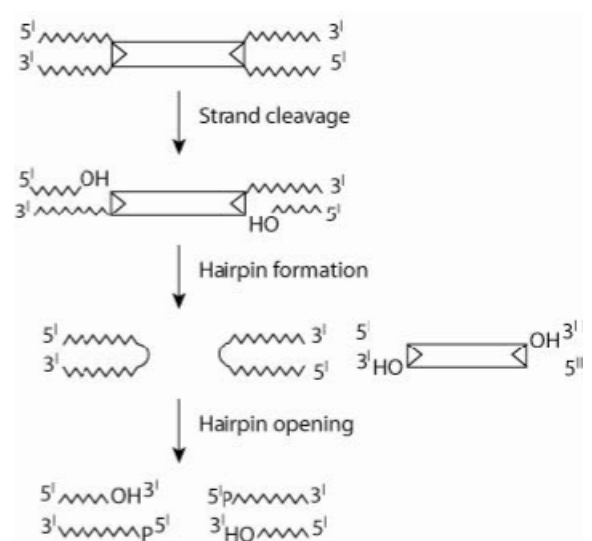
regions is often imprecise, involving additions, deletions etc. This adds to the coding diversity of the rearranged immunoglobin genes.

Multiple mechanisms for generating antibody diversity

The first mode of generating diversity is V(D)J recombination. Depending on the number of different V, D and J segments, diversity can be generated in a combinatorial fashion.

The opening of the hairpin coding ends is not always precise. If a 5' stagger is generated during the opening event, complementary bases can be added to the 3' end by a filling-in reaction by DNA polymerase.

The enzyme terminal nucleotidyl transferase also contributes to diversity. It adds nucleotides in a non-templated manner to the 3' end of the opened hairpin.

The additional bases incorporated introduce new amino acids in the hyper-variable region to expand the diversity of the antibody pool.

Note: Since there is no strict control of how many new bases are added at the coding ends, not all joined products will be in the right frame for translation. Only those that generate in-frame joining will result in functional heavy chain proteins. Thus, there is quite a bit of waste in the generation of functional antibodies. Nevertheless, the waste is worthwhile.

When a B cell, with a rearranged immunoglobin gene, is stimulated by an antigen, it goes into proliferative mode. During this process, the gene acquires mutations at a higher rate than spontaneous mutations (somatic hypermutations), particularly in the V region. These mutations generate B cell populations with distinct antigen recognition specificities. One of the enzymes involved in generating somatic mutations is activation induced cytosine deaminase (AID). The deamination of cytosine generates uracil in DNA. If unrepaired, during replication it will pair with A. Repair of the UA pair to the normal T-A pair will generate a mutation from the original C-G pair. Normally, repair enzymes will remove the uracil base by cutting the DNA strand on either side of it. The gap is filled in by error prone polymerases that generate mismatches. Subsequent replication will then result in mutations.

In some organisms, for example chickens, the main source of diversity is gene conversion events. In addition to the functional loci for antibody gene rearrangement, chickens contain a cluster of pseudogenes (non-functional genes) corresponding to V regions. However they serve

as templates for gene conversion. That is, by recombination mechanisms, the functional genes copy the sequences from these pseudogenes to generate diversity.