

A Prototype Ig Gene: Murine Kappa

About 100 V_{κ} gene segments 4 J Gene Segment s 1 C_{κ} 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

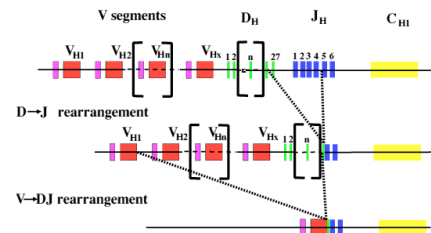
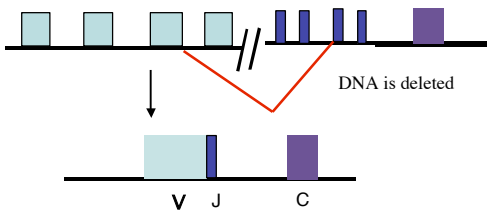
Generates Diversity

Class switch recombination:
heavy chains only

Changes Isotype (antigen elimination)

V(D)J Recombination in the Kappa Locus

About 100 V_{κ} gene segments 4 J Gene Segment s 1 C_{κ} Gene Segment



DNA Rearrangement Removes Sequences Between V, D and J Segments

RNA Splicing Removes Sequences Between J and C Segments

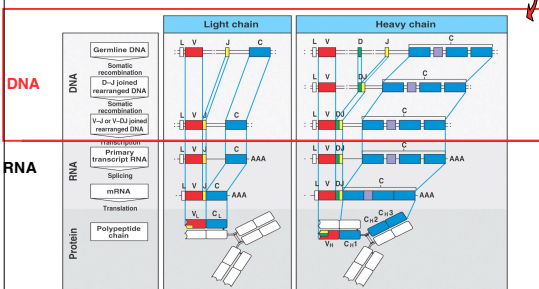
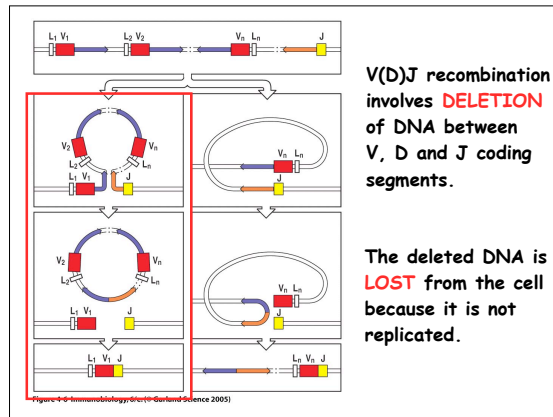


Figure 4-2 Immunobiology, 6/e. © Garland Science 2005



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

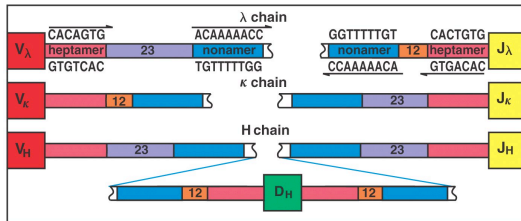
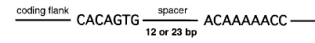
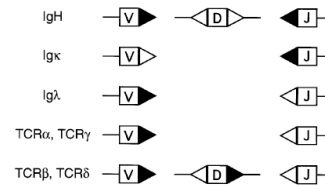


Figure 4-5 Immunobiology, 6/e. (© Garland Science 2005)

A



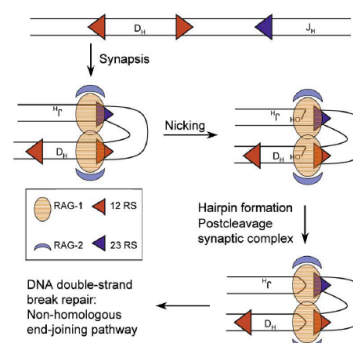
B



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:

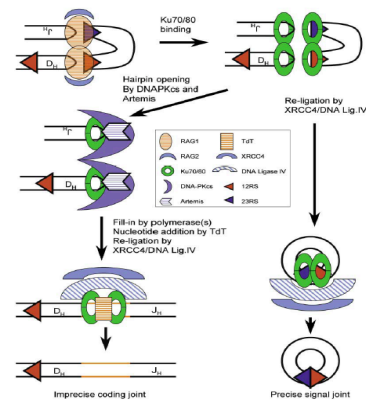
Ku70/80

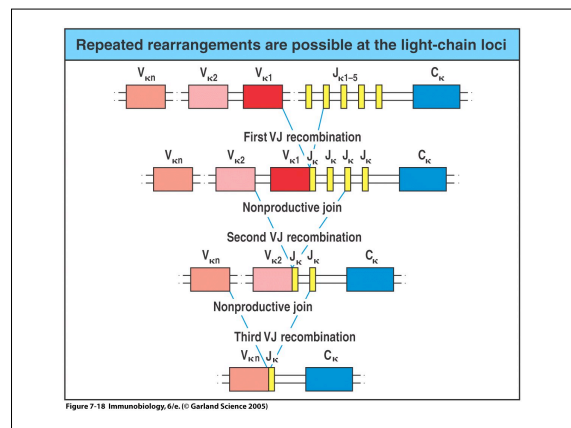
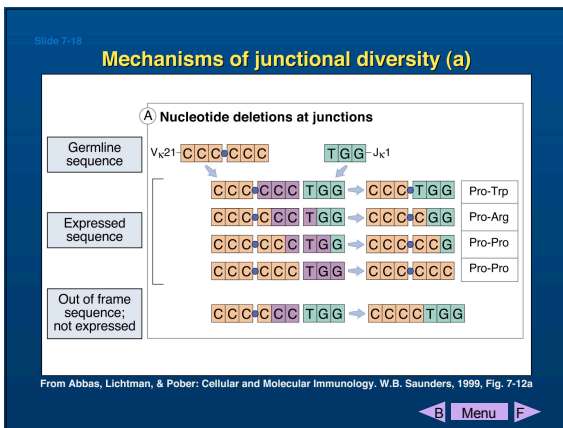
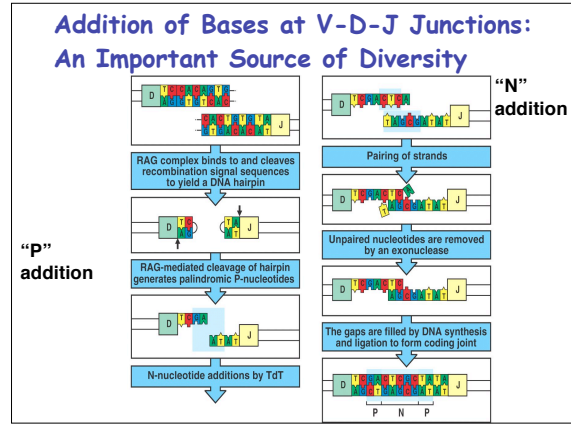
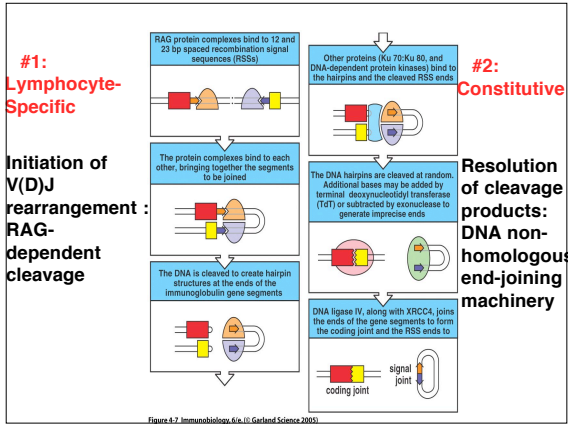
DNA-dependent protein kinase

Artemis

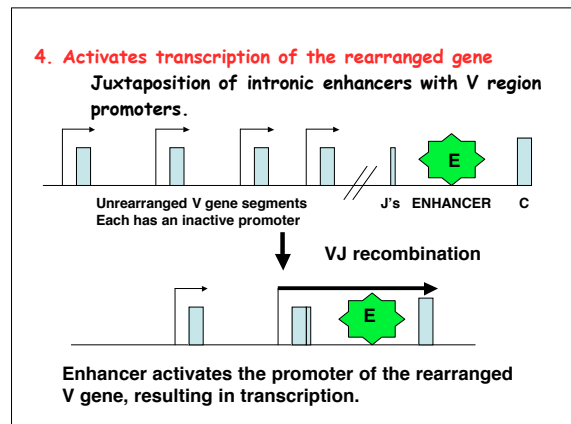
XRCC4

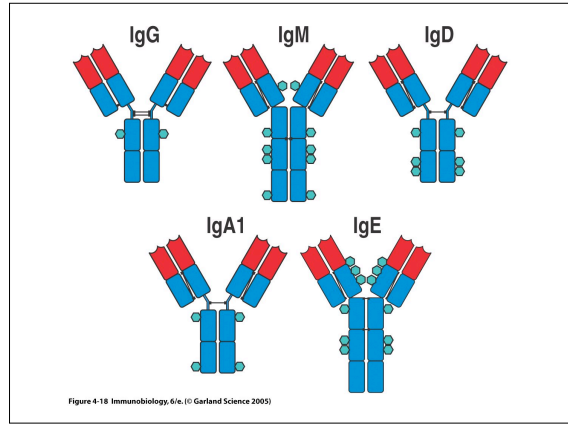
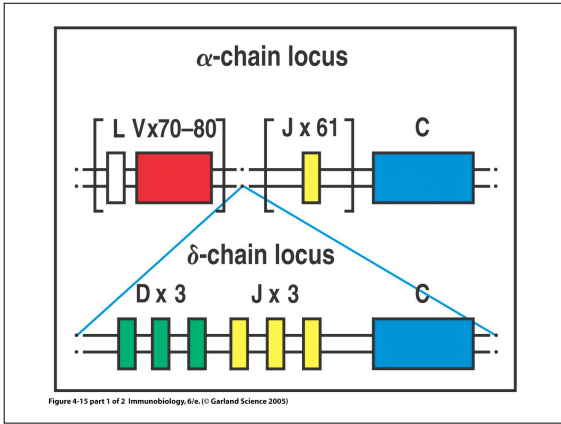
DNA ligase IV





- 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. Allows receptor editing** to alter potentially self-reactive antibodies





	Immunoglobulin								
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	γ ₁	γ ₂	γ ₃	γ ₄	μ	α ₁	α ₂	δ	ε
Molecular weight (kDa)	146	146	165	146	970	160	180	184	188
Serum level (mean adult mg ml ⁻¹)	9	3	1	0.5	1.5	3.0	0.5	0.03	5 × 10 ⁻⁴
Half-life in serum (days)	21	20	7	21	10	6	6	3	2
Classical pathway of complement activation	++	+	+++	-	+++	-	-	-	-
Alternative pathway of complement activation	-	-	-	-	-	+	-	-	-
Placental transfer	+++	+	++	-	-	-	-	-	-
Binding to macrophage and phagocyte Fc receptors	+	-	+	+	-	+	+	+	+
High-affinity binding to mast cells and basophils	-	-	-	-	-	-	-	-	+++
Reactivity with staphylococcal Protein A	+	+	-	+	-	-	-	-	-

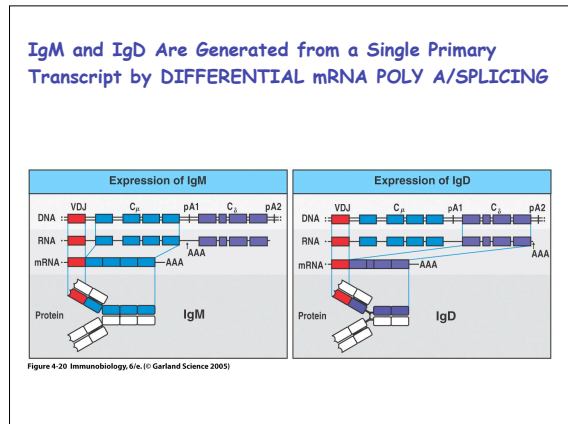
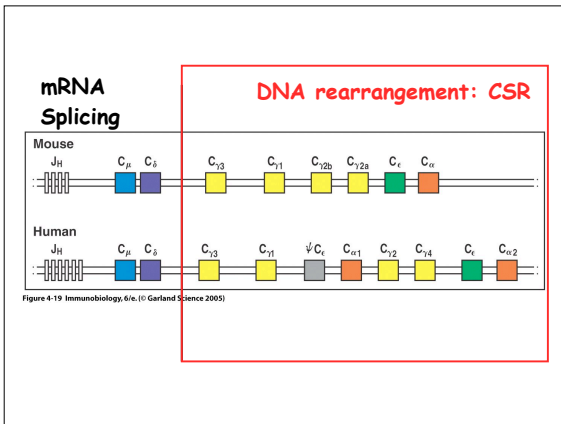
Figure 4-17 Immunobiology, 6/e. (© Garland Science 2005)

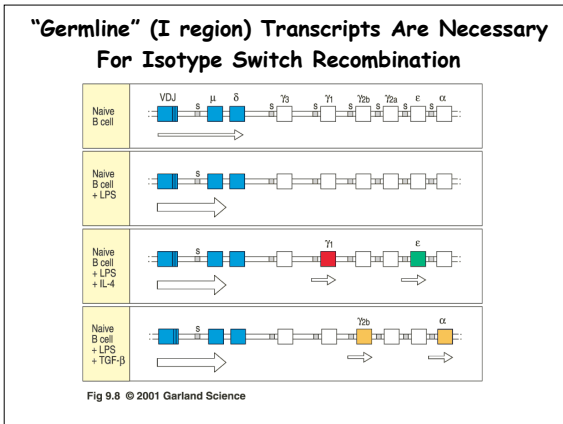
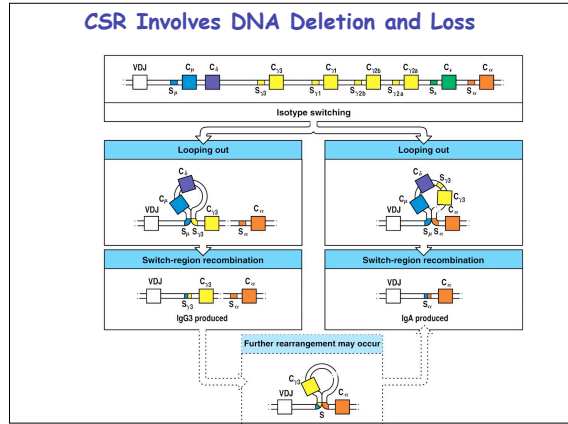
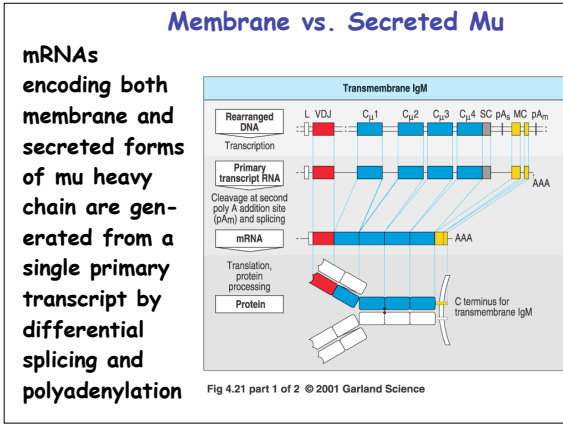
Ig HEAVY CHAIN LOCUS

1. Membrane vs secreted exons
2. Mu and delta isotypes
3. Gamma, epsilon and alpha isotypes

RNA

CLASS SWITCH RECOMBINATION (CSR)
A second DNA rearrangement, unique to the HC locus





Cytokines regulate I region transcription:

Role of cytokines in regulating Ig isotype expression							
Cytokines	IgM	IgG3	IgG1	IgG2b	IgG2a	IgE	IgA
IL-4	Inhibits	Inhibits	Induces		Inhibits	Induces	
IL-5							Augments production
IFN-γ	Inhibits	Induces	Inhibits		Induces	Inhibits	
TGF-β	Inhibits	Inhibits		Induces			Induces

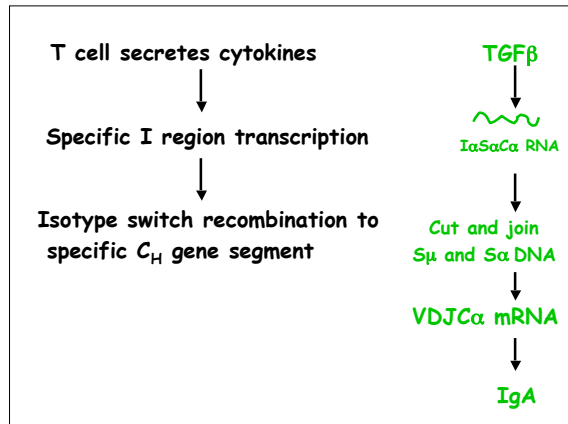
Figure 9-7 Immunobiology, 6/e. (© Garland Science 2005)

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



V(D)J Recombination	CSR
Join in exon	Join in intron
RAGs required	RAGs Not required AID is required
Repair enzymes	Repair enzymes
Generates diversity Ag specificity	Changes isotype 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