

A Prototype Ig Gene: Murine Kappa

About 100
V_κ gene segments

4 J Gene
Segments

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

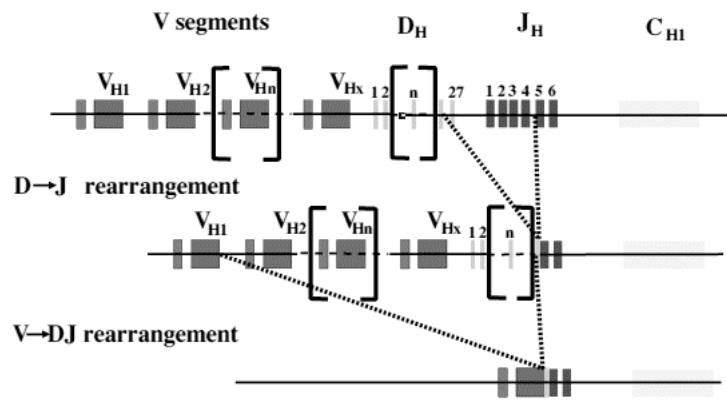
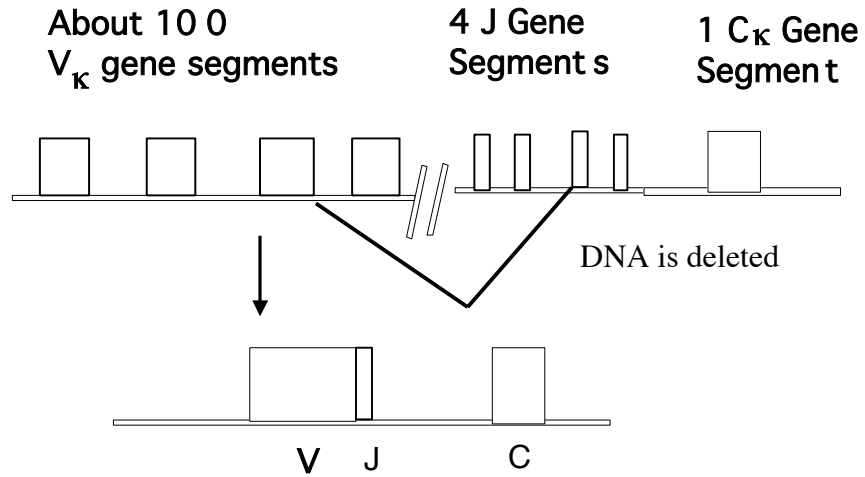
V(D)J Recombination:
both light and heavy chains

Generates
Diversity

Class switch recombination:
heavy chains only

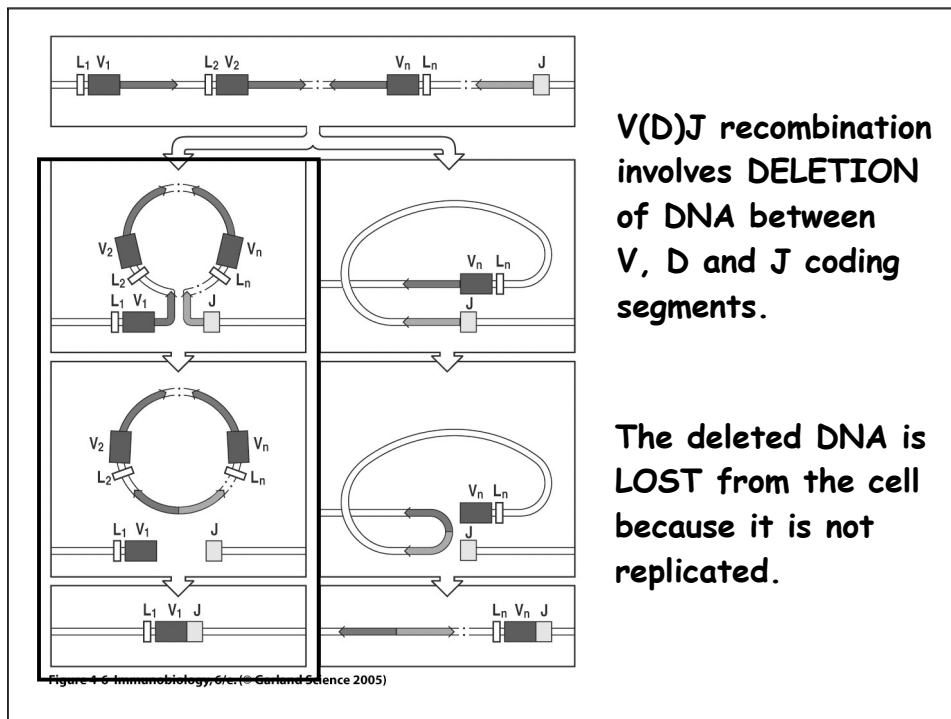
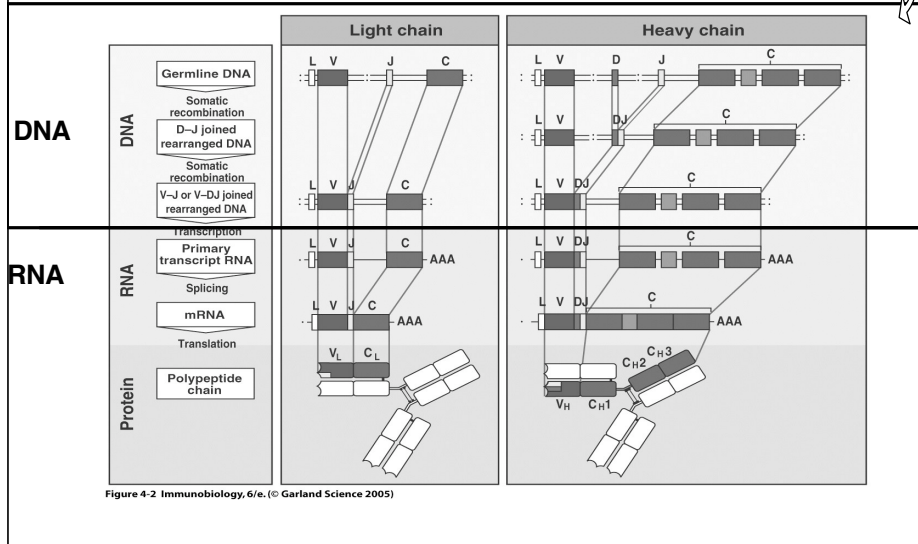
Changes
Isotype (antigen
elimination)

V(D)J Recombination in the Kappa Locus



DNA Rearrangement Removes Sequences Between V, D and J Segments

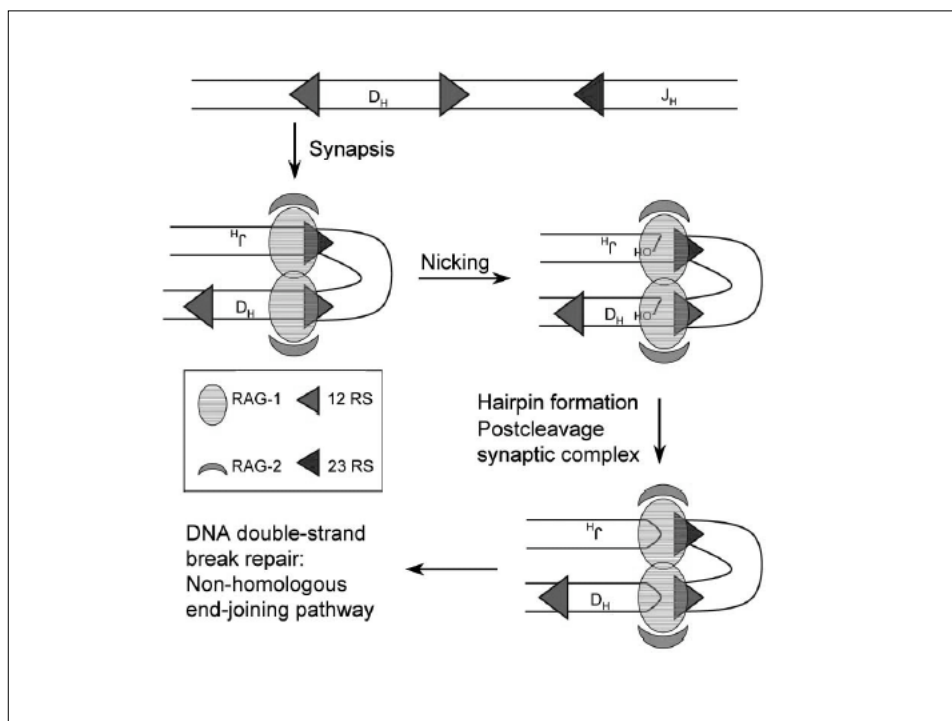
RNA Splicing Removes Sequences Between J and C Segments



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:

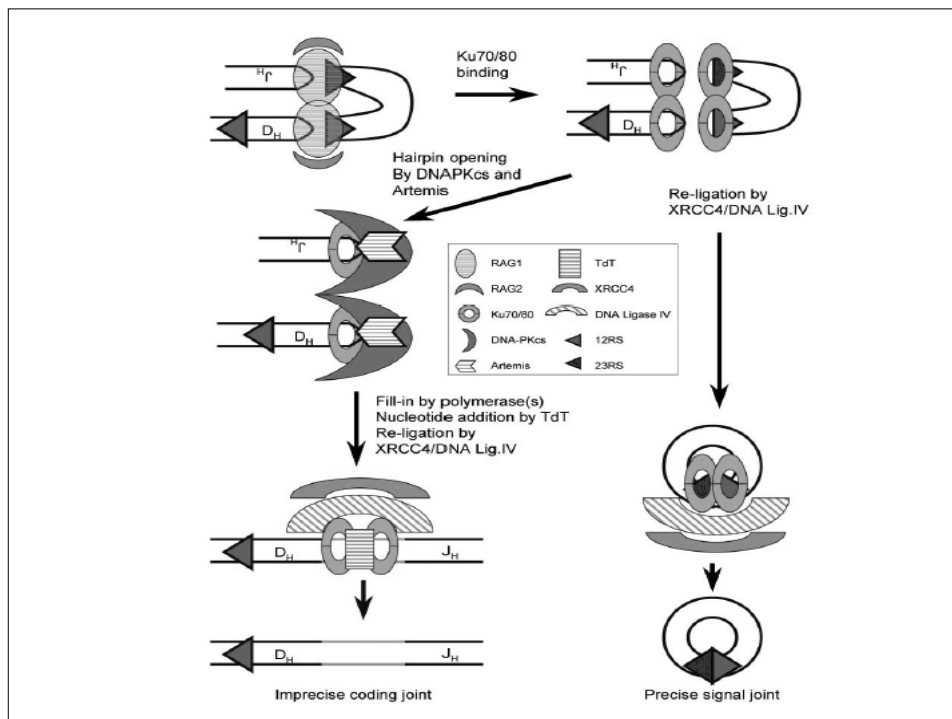
Ku70/80

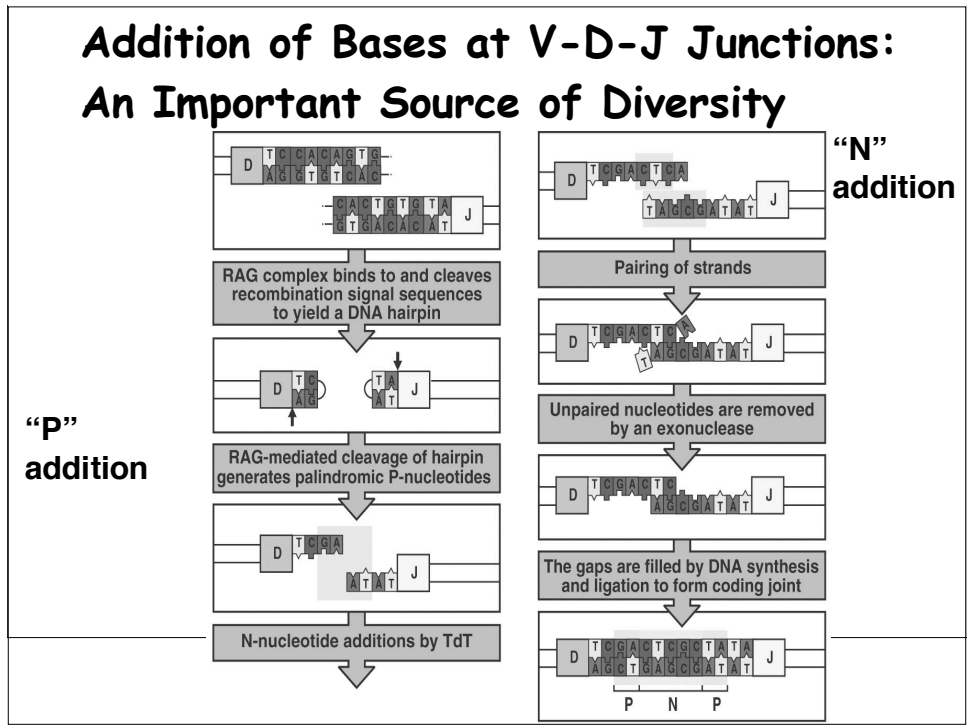
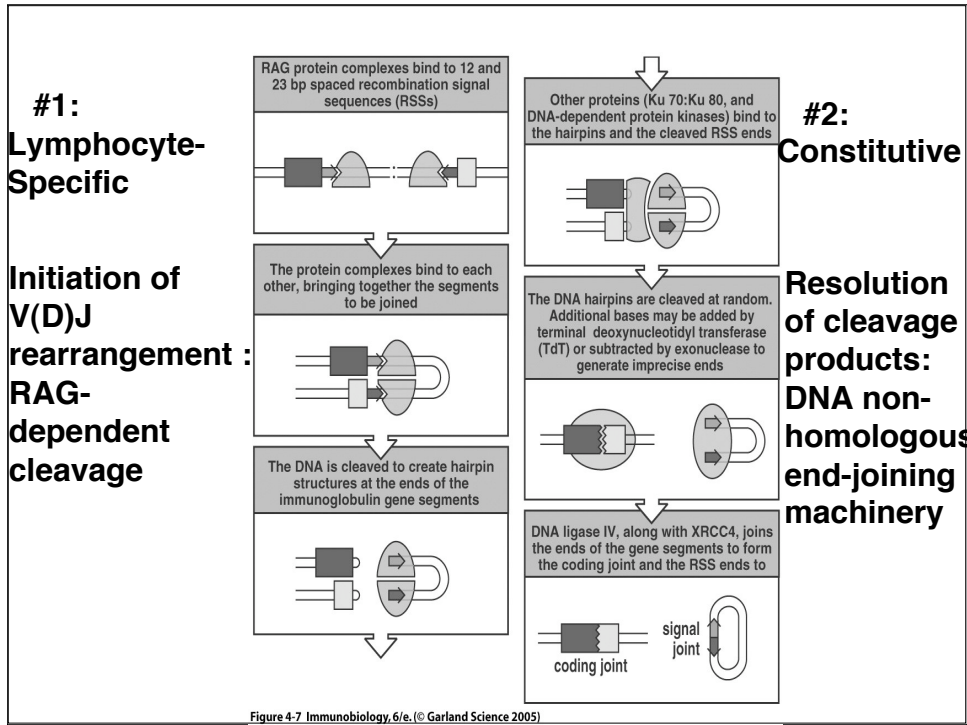
DNA-dependent protein kinase

Artemis

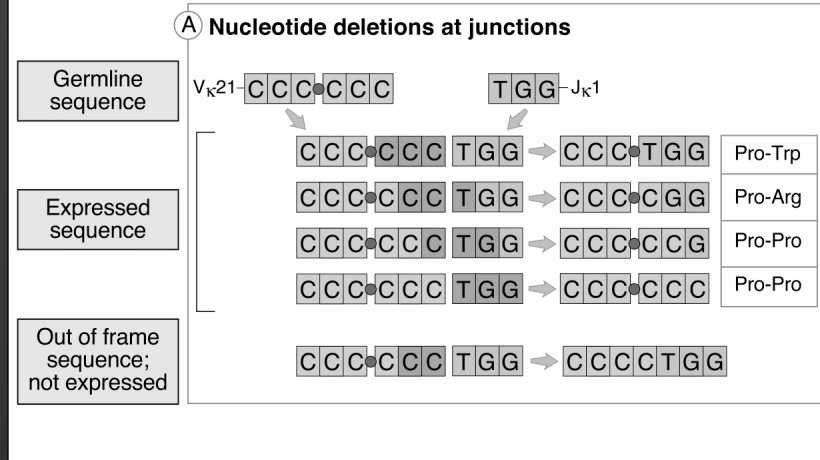
XRCC4

DNA ligase IV





Mechanisms of junctional diversity (a)



From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 7-12a

Repeated rearrangements are possible at the light-chain loci

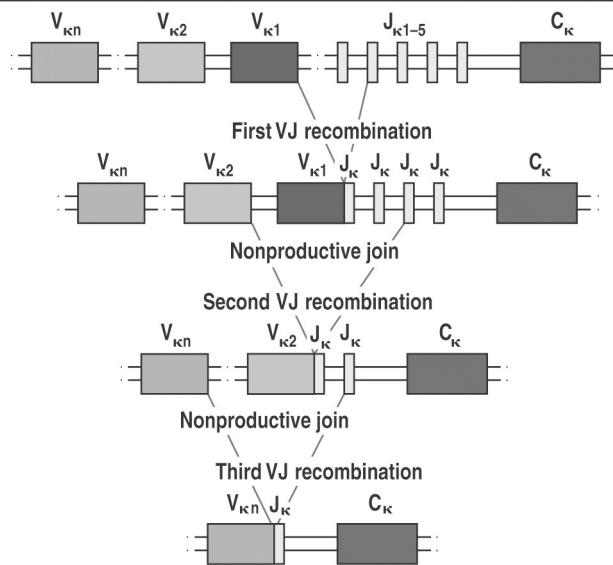
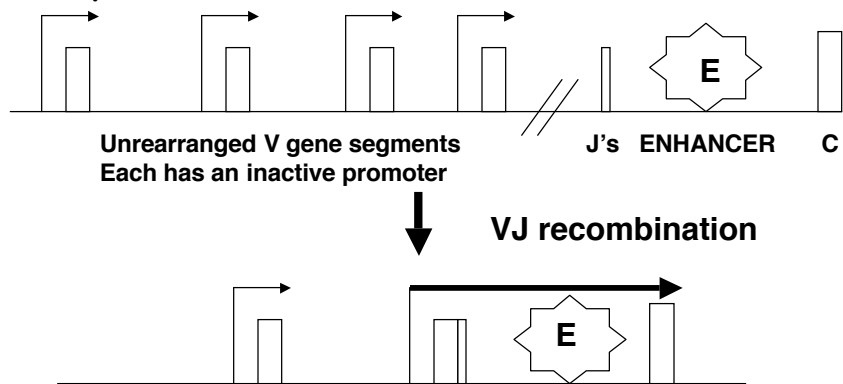


Figure 7-18 Immunobiology, 6/e. (© Garland Science 2005)

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**

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.

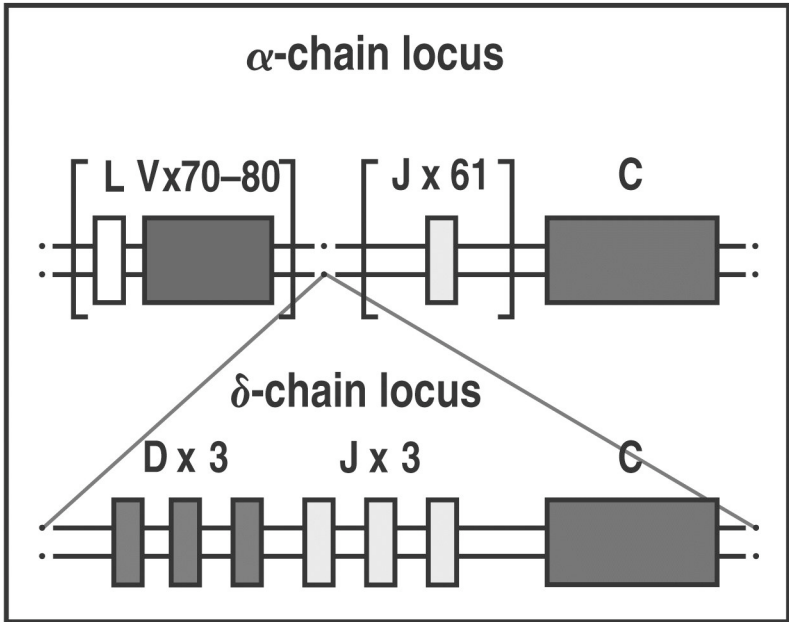


Figure 4-15 part 1 of 2 Immunobiology, 6/e. (© Garland Science 2005)

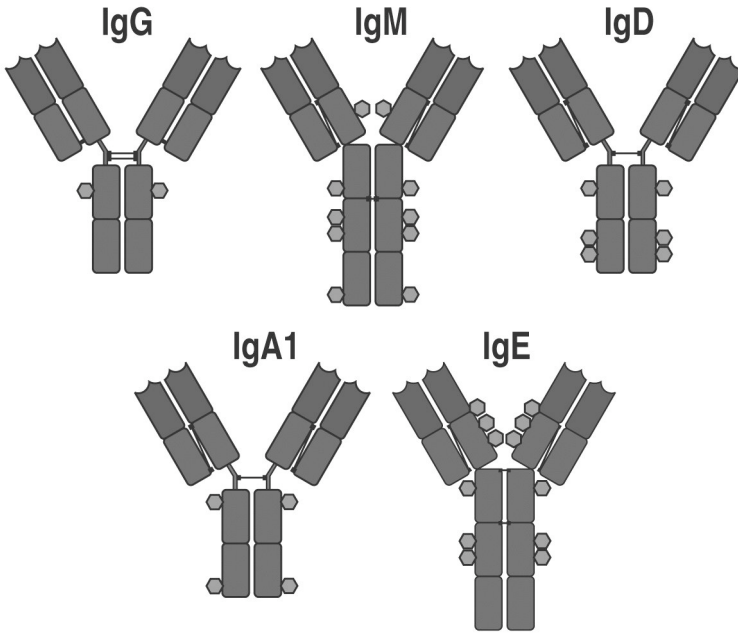


Figure 4-18 Immunobiology, 6/e. (© Garland Science 2005)

	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	160	184	188
Serum level (mean adult mg ml ⁻¹)	9	3	1	0.5	1.5	3.0	0.5	0.03	5×10^{-5}
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

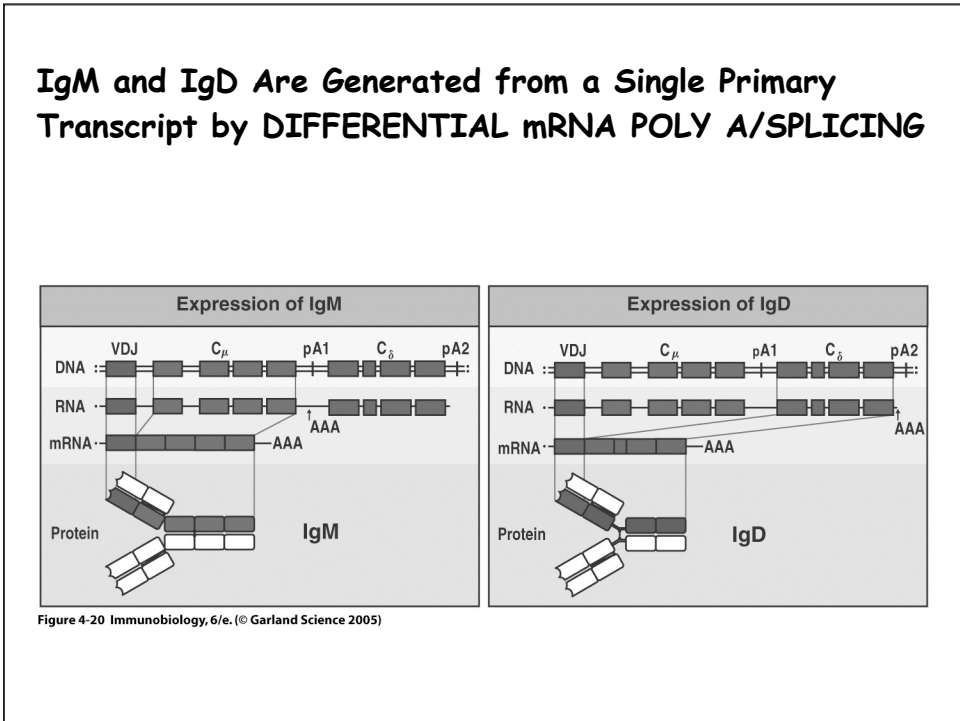
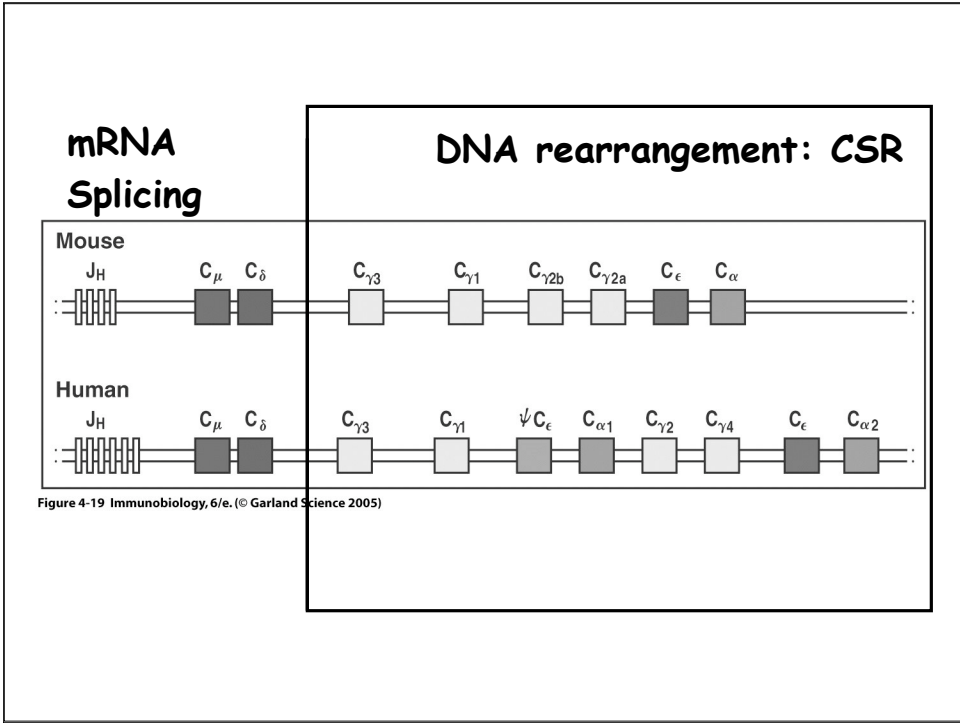
1. Membrane vs secreted exons

RNA

2. Mu and delta isotypes

3. Gamma, epsilon and alpha isotypes

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



Membrane vs. Secreted Mu

mRNAs encoding both membrane and secreted forms of mu heavy chain are generated from a single primary transcript by differential splicing and polyadenylation

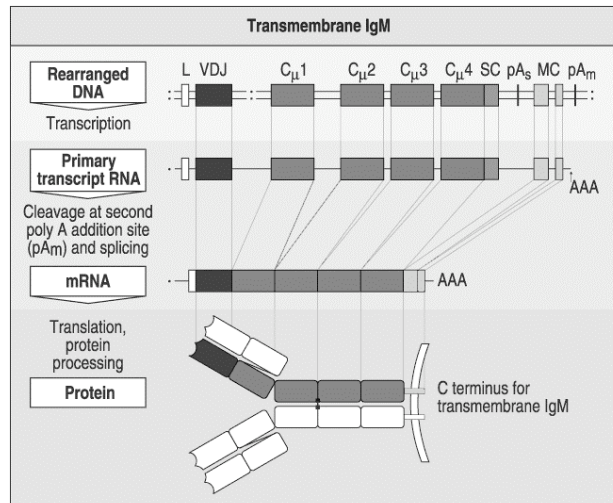
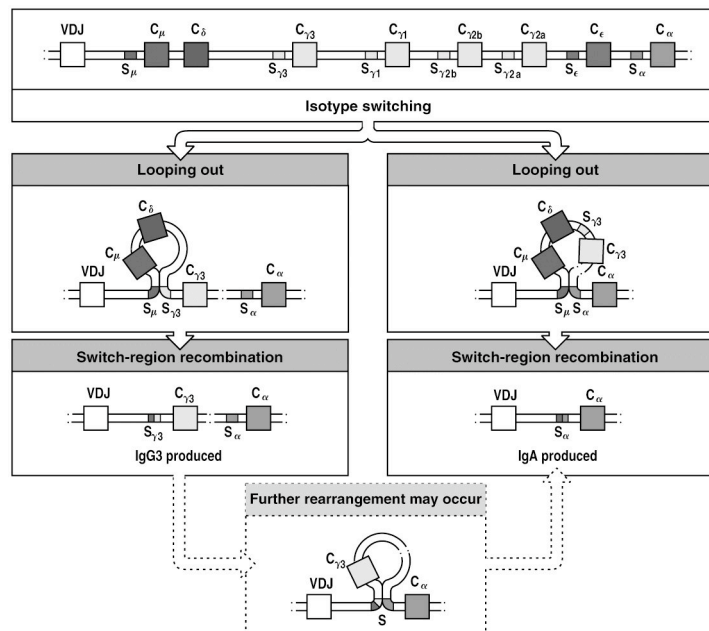


Fig 4.21 part 1 of 2 © 2001 Garland Science

CSR Involves DNA Deletion and Loss



"Germline" (I region) Transcripts Are Necessary For Isotype Switch Recombination

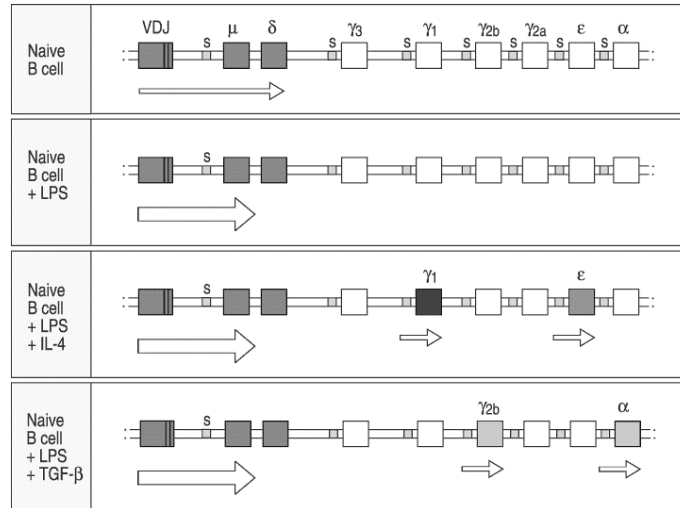


Fig 9.8 © 2001 Garland Science

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)

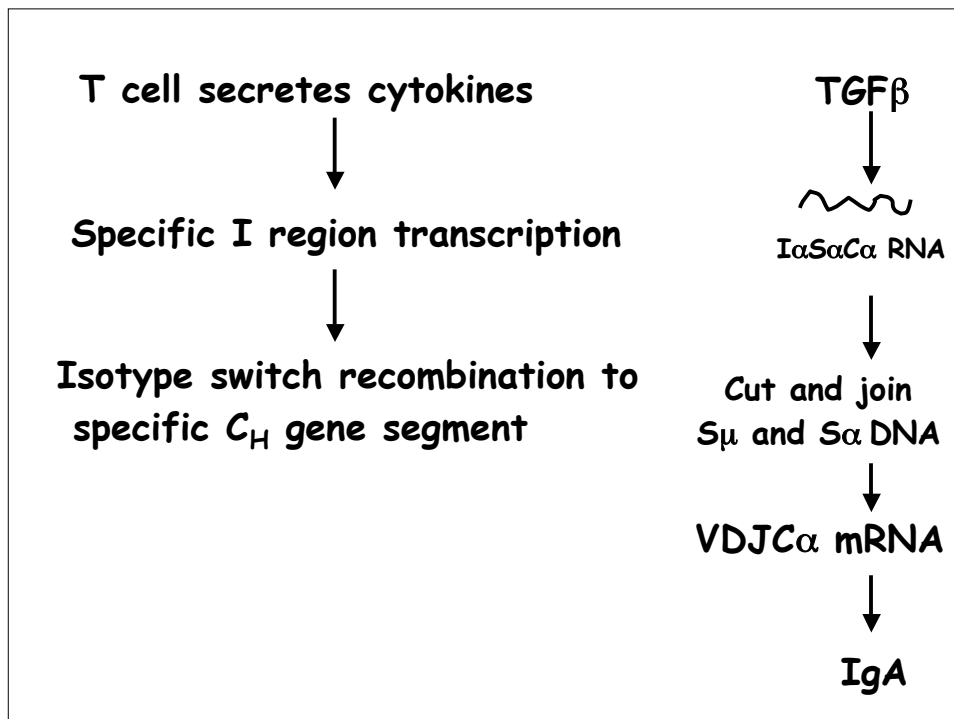
AID (Activation Induced Deaminase) is required

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