Ig Polypeptides Are Encoded by Multiple Gene Segments

LIGHT CHAIN

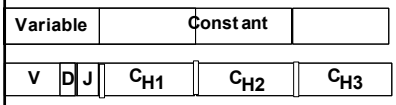
Light Chain POLYPEPTIDE

Polypeptide

Gene Segments

Light Chain GENE SEGMENTS

HEAVY CHAIN



H.C. POLYPEPTIDE

Polypeptide

Gene Segments

H.C. GENE SEGMENTS

Human Ig Loci

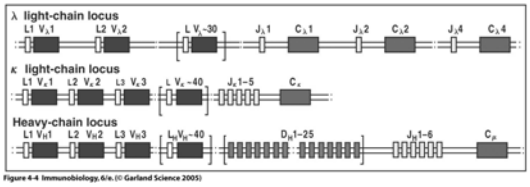


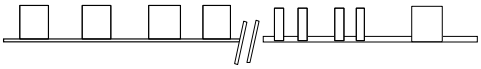
Figure 4-4 Immunobiology, 6/e. (© Garland Science 2005)

A Prototype Ig Gene: Murine Kappa

About 10 0 V_κ gene segments

4 J Gene Segment s

1 C_κ Gene Segment

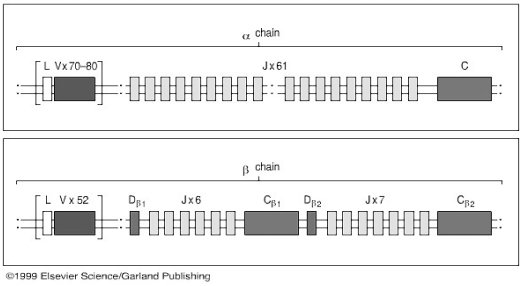


Multiple V gene segments, distant from J and C

A few J gene segments

One C gene segment

TCR Alpha and Beta Loci



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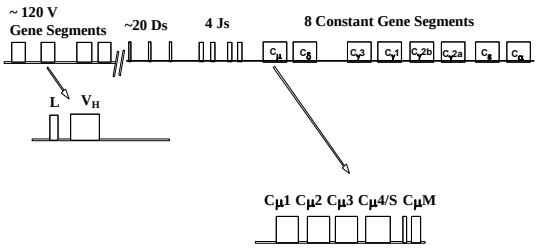
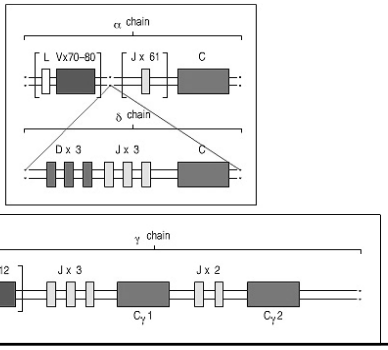
Murine Ig Heavy Chain Gene Organization



TCR Delta and Gamma Loci



IMMUNOGLOBULIN GENES UNDERGO *TWO* DNA REARRANGEMENTS

1. V(D)J Recombination: both light and heavy chains
2. Class switch recombination: heavy chains only

Recombination Signal Sequences (RSSs) Flank Rearranging Gene Segments

RSS=heptamer, spacer and nonamer

7bp → 12/23 bp 9bp →

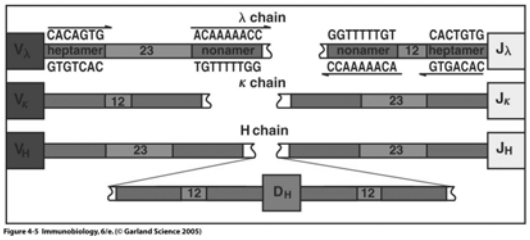


Figure 4-5 Immunobiology, 6/e. (© Garland Science 2005)

DNA Rearrangement RemovesSequences Between V, D and J Segments

RNA Splicing Removes Sequences Between J and C Segments

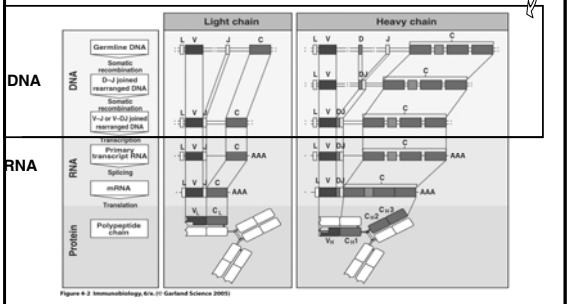
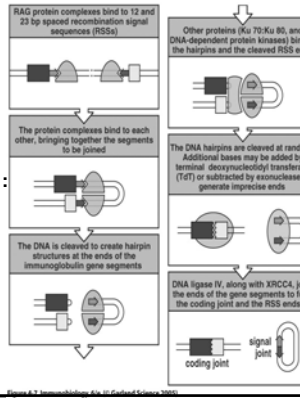


Figure 4-2 Immunobiology, 6/e. (© Garland Science 2005)

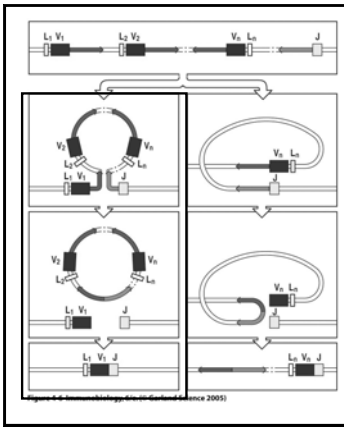
#1: Lymphocyte-Specific

Initiation of V(D)J rearrangement : RAG-dependent cleavage



#2: Constitutive

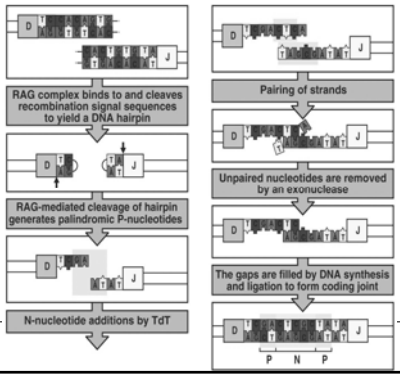
Resolution of cleavage products: DNA non-homologous end-joining machinery

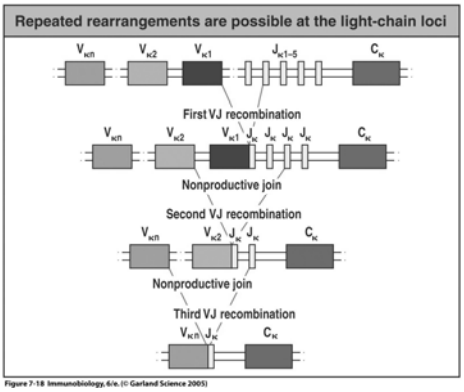


V(D)J recombination involves DELETION of DNA between V, D and J coding segments.

The deleted DNA is LOST from the cell because it is not replicated.

An Important Source of Diversity



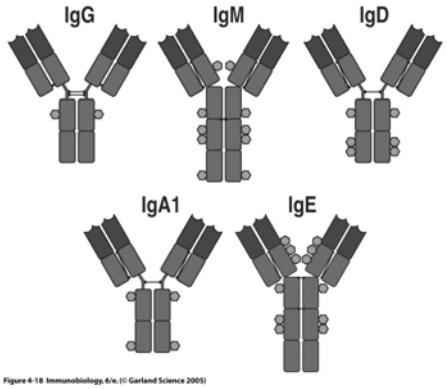


Heavy chain isotypes are generated by a second DNA rearrangement:

CLASS SWITCH RECOMBINATION (CSR)

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.: 120x20x4=10^4
2. Junctional diversity at CDR3
   - Deletion of bases at junctions
   - N region additions at junctions
   - P region additions at junctions
3. Activates transcription of the rearranged gene
   - Juxtaposition of intronic enhancers with V region promoters.
4. Allows receptor editing to alter potentially self-reactive antibodies



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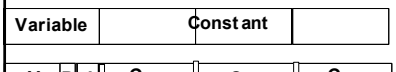
LIGHT CHAIN

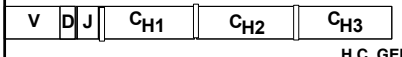
Light Chain POLYPEPTIDE

Light Chain GENE SEGMENTS

HEAVY CHAIN



H.C. Polypeptide



Gene Segments

H.C. GENE SEGMENTS

	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)

mRNA Splicing

DNA rearrangement: CSR

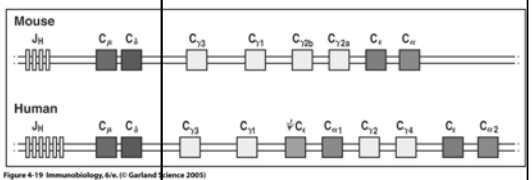
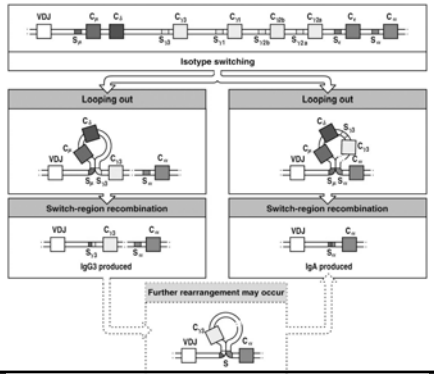


Figure 4-19 Immunobiology, 6/e. (© Garland Science 2005)

CSR Involves DNA Deletion and Loss



IgM and IgD Are Generated from a Single Primary Transcript by DIFFERENTIAL mRNA POLY A/SPLICING

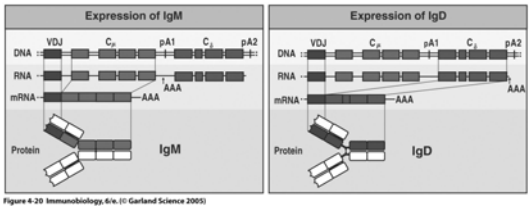


Figure 4-20 Immunobiology, 6/e. (© Garland Science 2005)

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

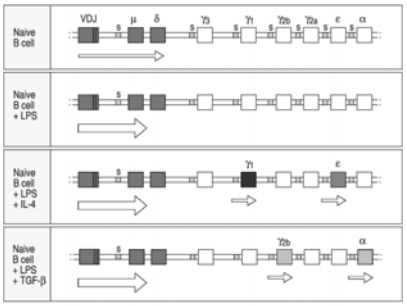


Fig 9.8 © 2001 Garland Science

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

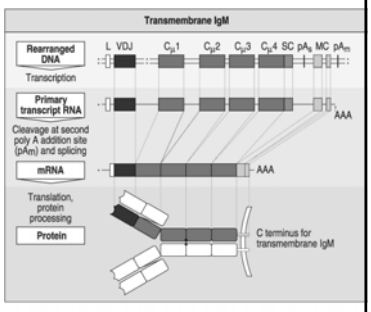
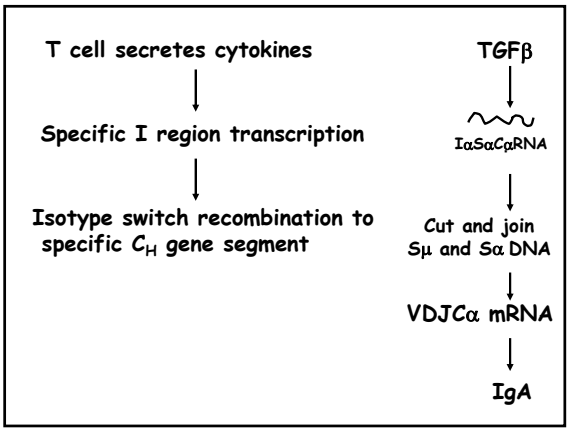


Fig 4.21 part 1 of 2 © 2001 Garland Science

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)



SUMMARY

1. Ig genes undergo two DNA rearrangements which result in loss of DNA: VDJ recombination and class switch recombination. TCR genes undergo VDJ recombination only.
2. VDJ recombination requires lymphocyte-specific RAG (recombination activating gene) proteins and ubiquitous double-strand break repair proteins.
3. VDJ recombination provides diversity thru recombinational mechanisms and junctional diversity; it also activates gene transcription.
4. CSR occurs in introns and requires AID (activation induced cytidine deaminase).
5. CSR allows changes in the heavy chain isotype, leading to different antigen elimination properties of the expressed antibody.
6. Defects in genes encoding RAG, AID and other factors cause human immune deficiency diseases.

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