Antibodies

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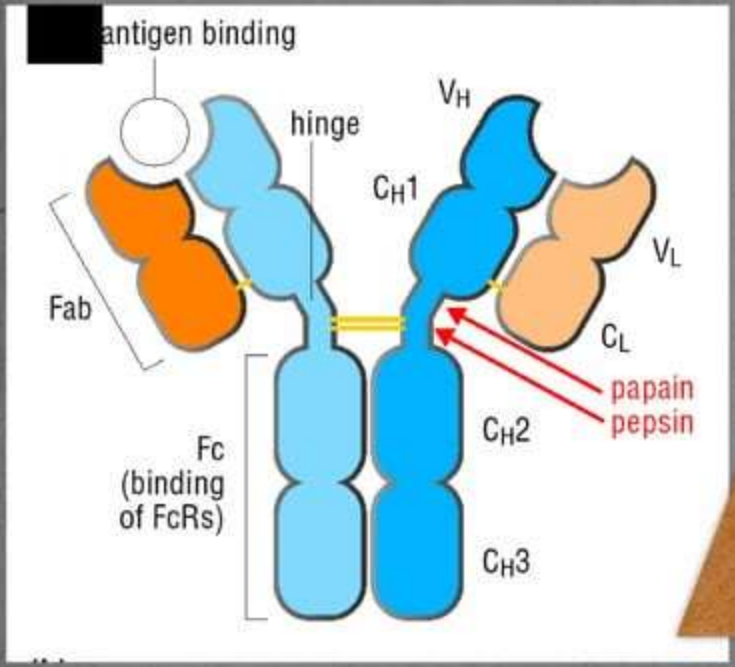
Antibodies

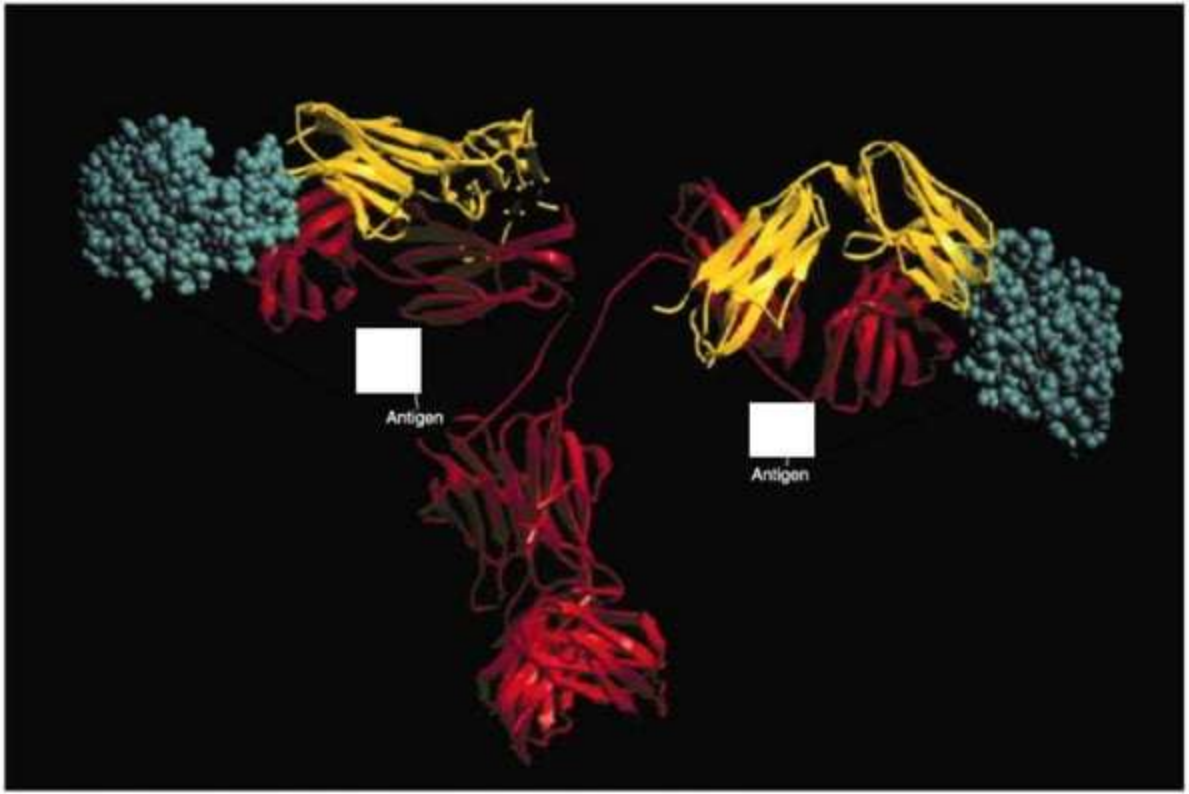
- Secreted by B lymphocytes
- Great diversity and specificity: $>10^9$ different antibodies; can distinguish between very similar molecules
- Tag particles for clearance/destruction
- Protect against re-infection (vaccines)

Antibody Structure



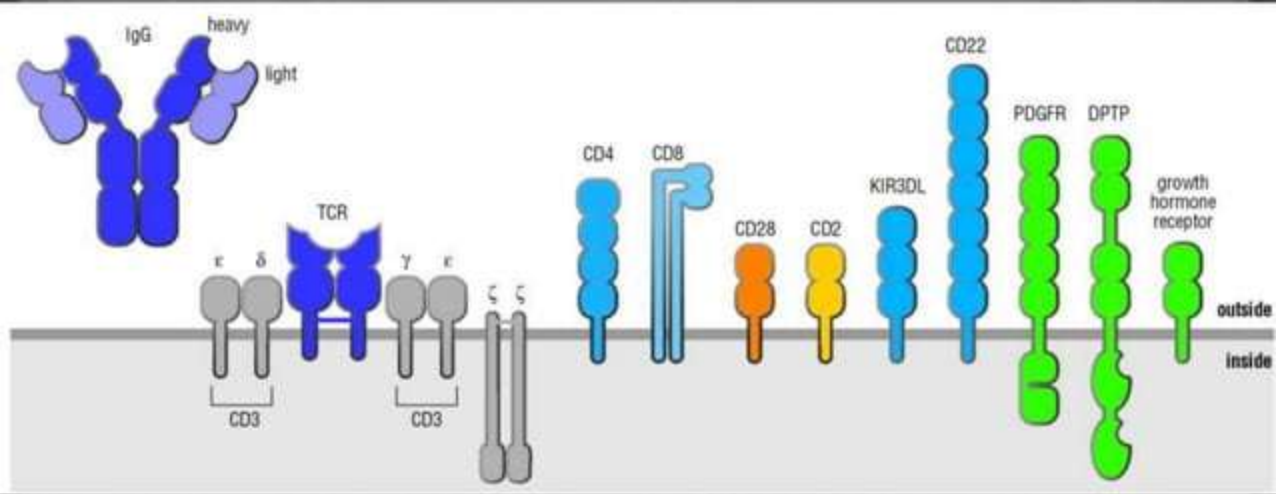
- Ig domain: 110 amino acids; globular domain used in many proteins.
- Variable domains,
- Constant domains, Hinge.
- Fab: fragment antigen binding
- Fc: fragment crystallizable (effector functions)

Binding of an antigen by an antibody.



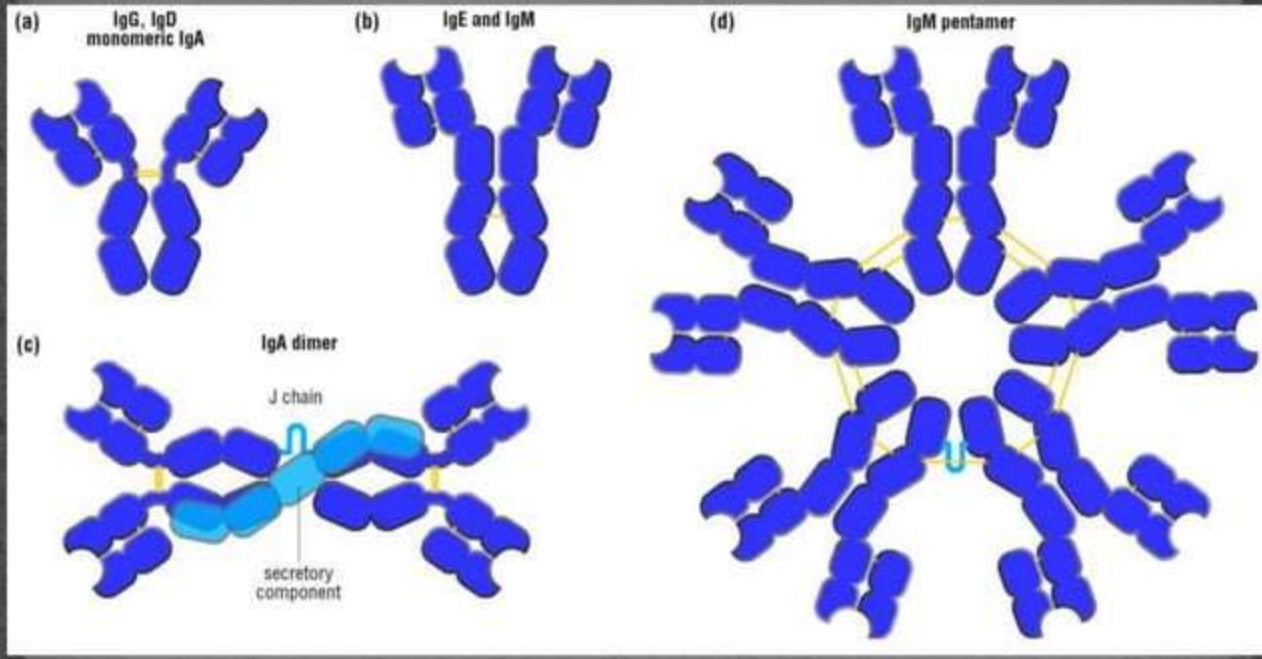
The Immunoglobulin Superfamily

a few examples



Variability in antibodies is clustered in the loops in the variable domains of the heavy and light chains (green); these regions are responsible for binding to antigen.

Antibody Classes: Structure



- Affinity and Avidity

•**Affinity**: the strength of binding between a single binding site and a single ligand.

$$K_D = \frac{[A][B]}{[AB]}$$

•**Avidity**: the strength of binding between a molecule and a complex ligand, e.g. if there are multiple binding sites then the avidity may be increased by increasing the number of binding sites or by increasing the affinity of those binding sites.

Affinity and Avidity, continued

IgM is produced early in an immune response when the affinity for antigen often is low; as an immune response continues, antibody affinity is improved, this is combined by "class switching" to the use of smaller molecules (IgG, IgE and IgA). The increased affinity compensates for the decrease in number of binding sites in maintaining the overall avidity for antigen.

Major functional properties of antibodies

Antibody class	Major Functional properties
IgM	complement activation; antigen trapping; antigen receptor of naïve B cells
IgG	complement activation, phagocytosis, ADCC, transfer of adaptive immunity to offspring, regulation of antibody production
IgA	mucosal immunity, phagocytosis
IgE	activation of mast cells, basophils, eosinophils
IgD	antigen receptor on naïve B cells

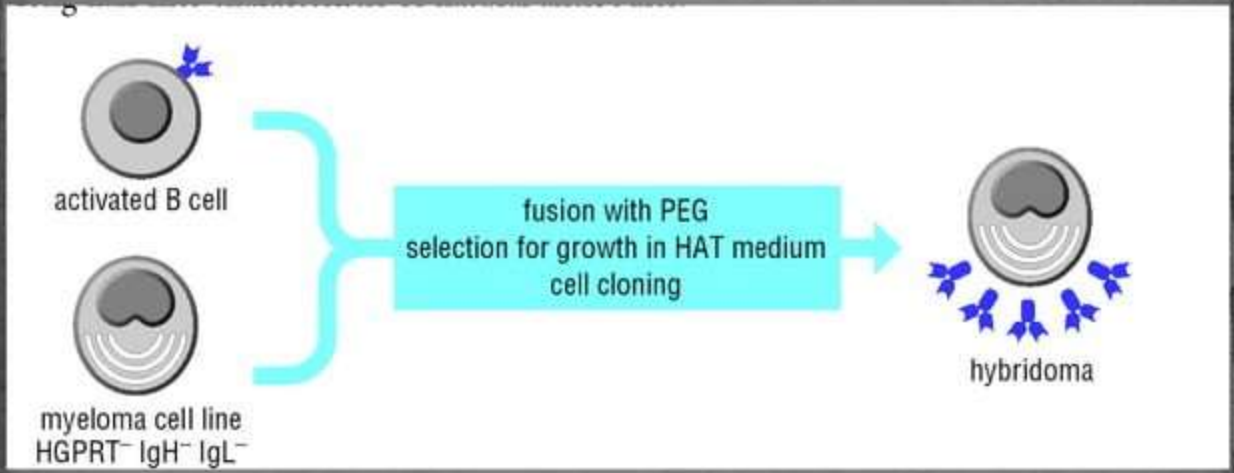
Monoclonal Antibodies

Single antibody (all same H and L chains)

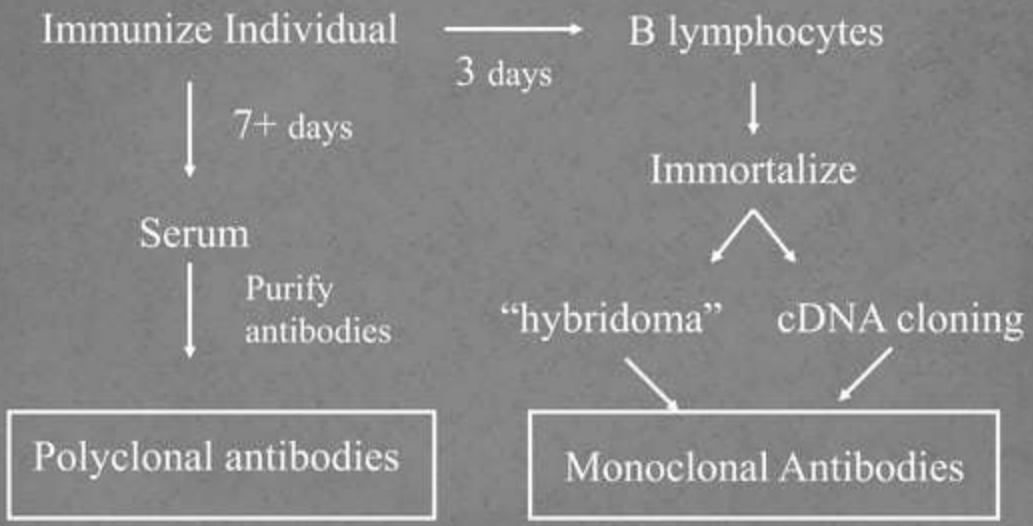
Made by fusion of B cells to a transformed cell line of the plasma cell type and selection for "hybridomas" that produce antibody with the desired properties

Standardized, unlimited reagent for diagnosis or therapy (human antibodies or "humanized" antibodies can be made)

Generation of Monoclonal Antibodies



Polyclonal vs. Monoclonal Antibodies



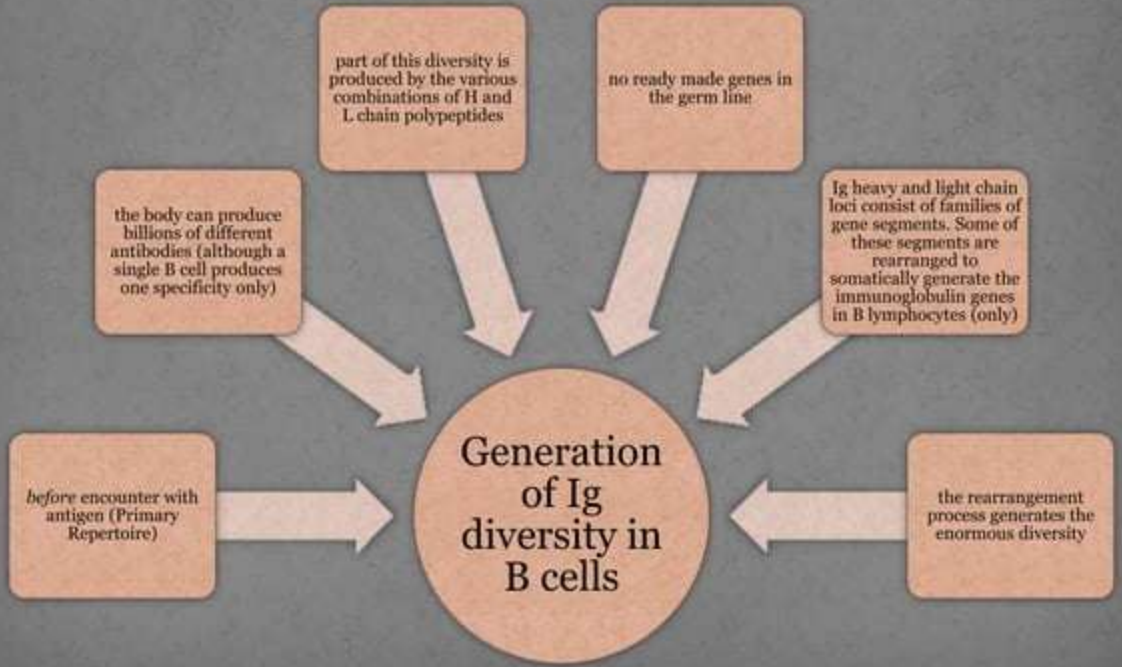
Monoclonal antibodies used in medicine

Standardized, unlimited amounts of reagents for diagnosis or therapy (human antibodies or "humanized" antibodies can be made).

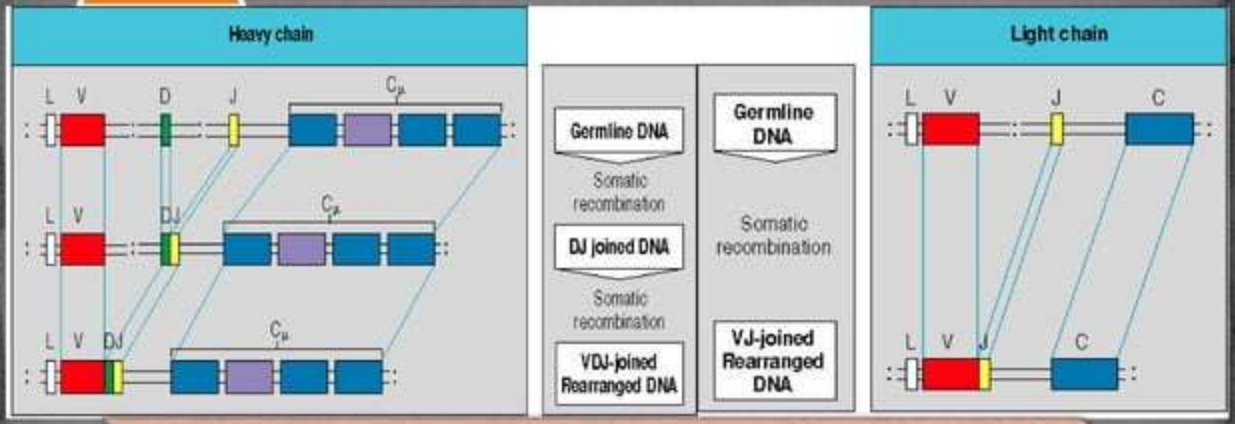
Monoclonal Antibodies Used in Therapies

monoclonal antibody	target	disease
trastuzumab	HER2	breast cancer
infliximab	TNF	rheumatoid arthritis, Crohn's disease
rituximab	CD20	non-Hodgkin's lymphoma
abciximab	GPIIb/IIIa	coronary disease
OKT3	CD3	graft rejection

Immunoglobulin genes



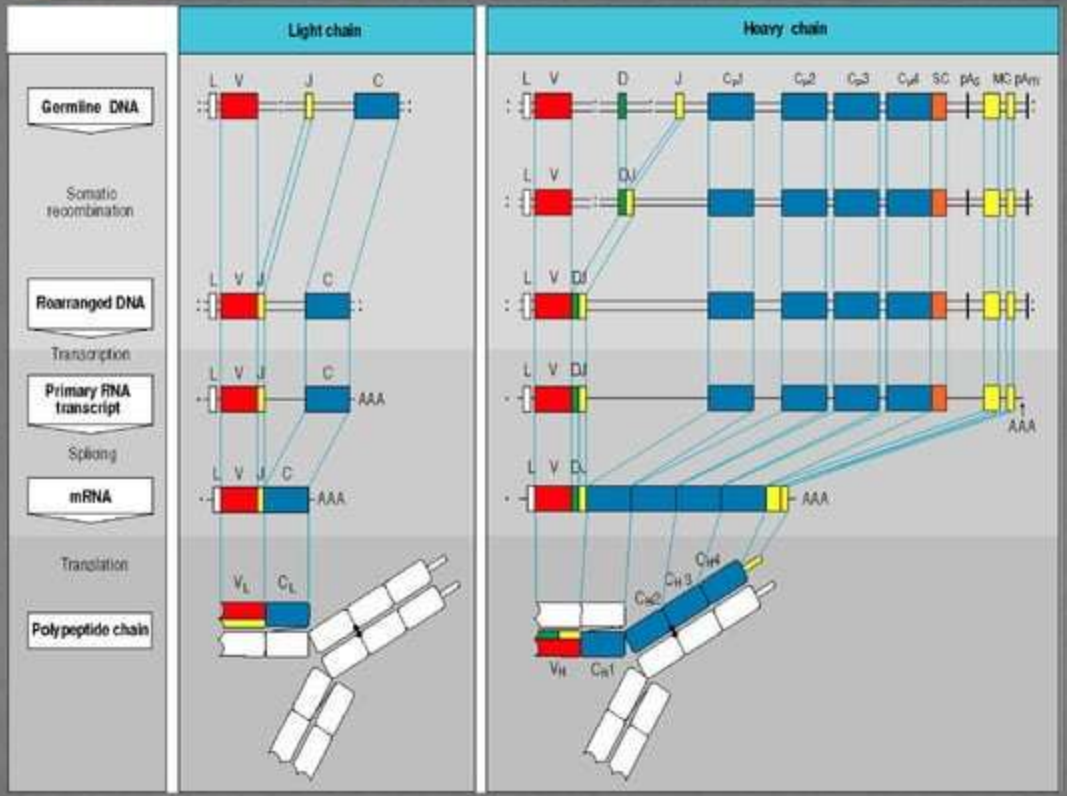
Recombination of gene segments produces a single gene encoding H chain, and a single gene encoding L chain in a given B lymphocyte



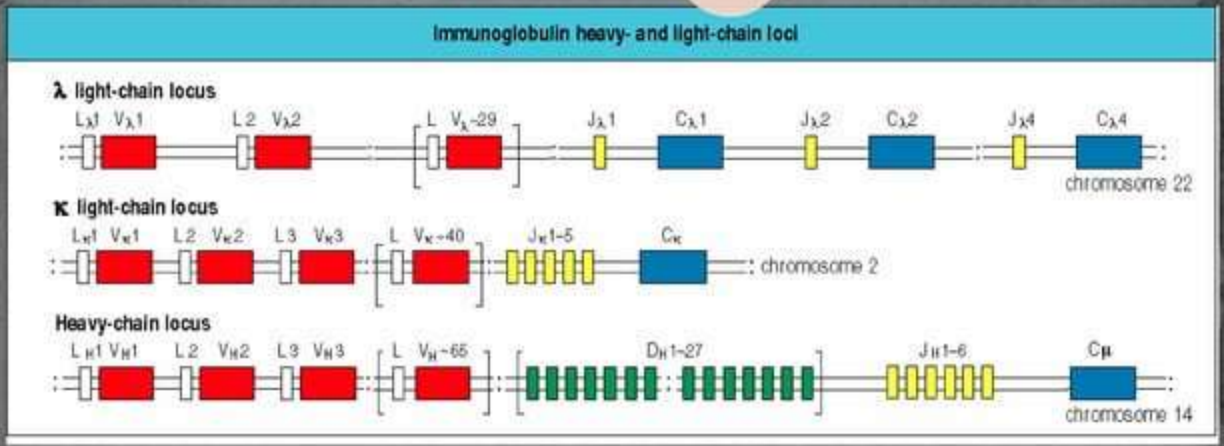
This unique process of DNA rearrangement happens (daily) when a hemopoetic stem cell differentiates into a B lymphocyte

Note: Unfortunately, the one gene segment that needs to be joined to the D or J segments is called V, similar to the V region of the H or L chain polypeptide. However, it encodes only part of the V polypeptide sequence.

Summary — Making IgM from DNA to RNA to Protein



- There are several copies of the V, D, and J segments, respectively. Different B cells choose different copies. This creates diversity in the antigen binding sites of antibodies generated by all B cells, even though an individual B cell produces only one kind of antibody.



Generation of Antibody Diversity

κ light chains: 40 Vκ x 5 Jκ = 200

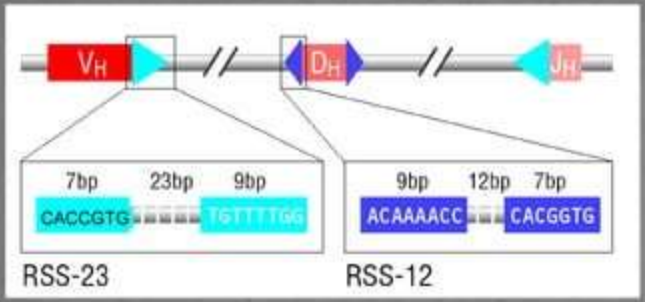
λ light chains: 30 Vλ x 4 Jλ = 120

H chains: 40 V_H x 27 D_H x 6J_H = 6,480

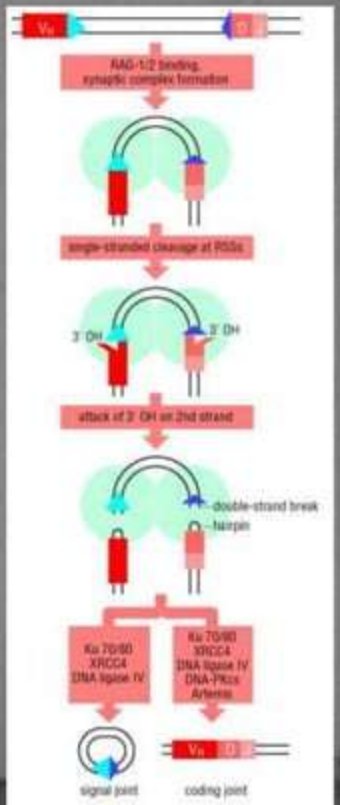
320 L chains x 6,480 H chains = 2.1 x 10^6

Junctional diversity (addition or deletion of nucleotides at recombination sites, especially of H chain), estimated to add 3x10^7 fold to overall diversity.

Mechanism of V(D)J recombination

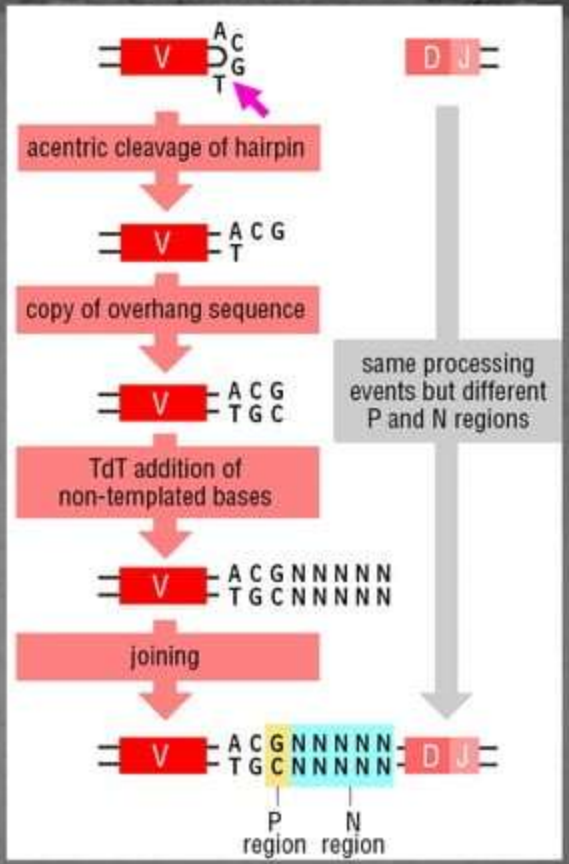


- Recombination signals
- Rag-1/Rag-2/Artemis
- Non-homologous end joining proteins
- defects: Severe Combined Immunodeficiency (SCID)



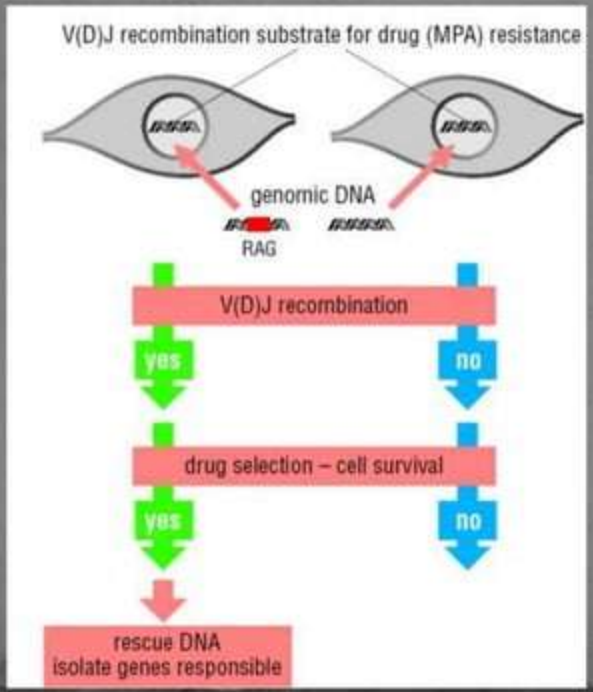
- Recombination enzymes produce additional diversity in the antigen-binding sites of Igs, termed *Junctional Diversity*

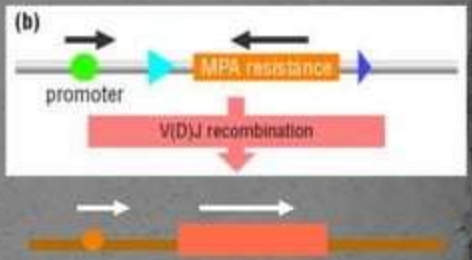
- Creation of Junctional Diversity by P-regions and TdT



Discovery of Rag1, 2 genes

"Recombination Activating Gene"





Defects in Lymphocyte development leading to severe combined immunodeficiency (SCID)

gene defect	result
RAG-1 or RAG-2	T^-B^- SCID
Artemis	T^-B^- SCID
γc cytokine receptor	X-linked SCID
JAK3	SCID

Note: SCID can also result from defects that interfere with lymphocyte activation (adenosine deaminase deficiency, purine nucleotide phosphorylase deficiency, MHC defects, etc.)

Allelic and isotypic exclusion

At each of the loci encoding Igs, only one (at most) of the two alleles is functional in any one lymphocyte; this is called allelic exclusion, and it ensures that all of the antibody molecules produced by a cell have the same specificity.

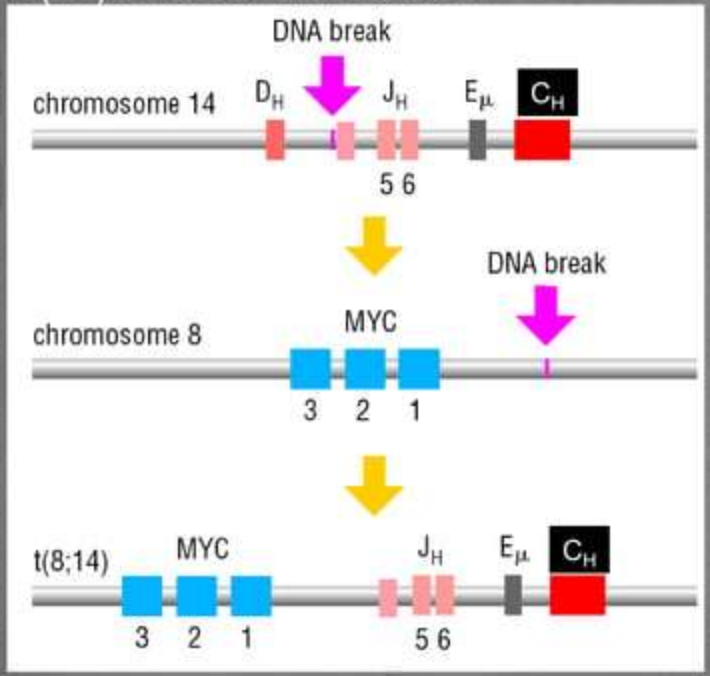
Furthermore, in a given lymphocyte, either κ or λ light chain, but not both, can combine with heavy chain to form a complete Ig molecule; this is called L chain isotypic exclusion.

Allelic and isotypic exclusion, continued

The precise mechanism for H chain allelic exclusion is not known, although a feedback mechanism generally is assumed.

The H genes are formed before the L genes. After a functional antigen receptor is formed, the RAG genes are turned off; this ends Ig gene rearrangement and mediates L chain allelic and isotypic exclusion.

Lymphoid malignancies resulting from errors in V(D)J recombination

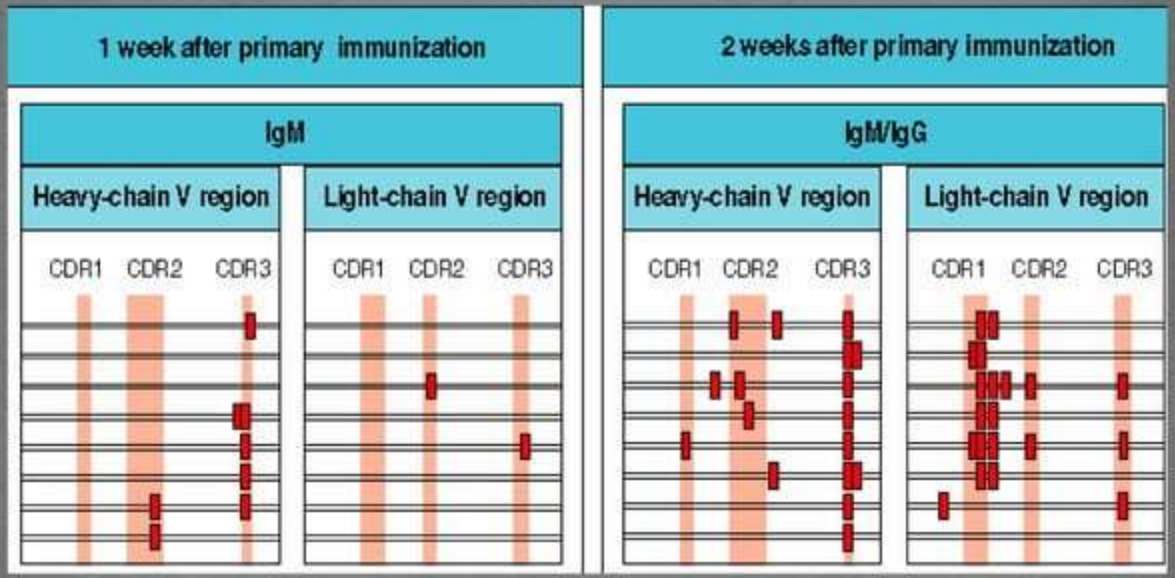


VDJ Recombination (and switch) reactions contribute to translocation leading to over-expression of a cellular growth or survival promoting gene

Generation of Ig diversity in B cells *after* encounter with antigen (Secondary Repertoire)

Rearranged V-region gene segments are further diversified by somatic hypermutation

This leads to antibodies with increased affinity for the inducing antigen



Red boxes - somatic mutations;
CDR, complementarity determining regions

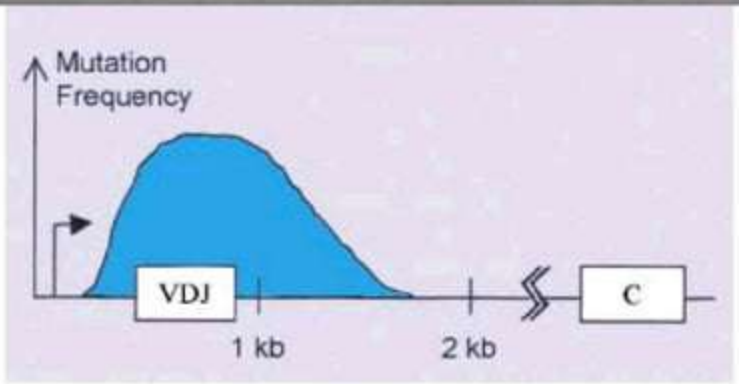
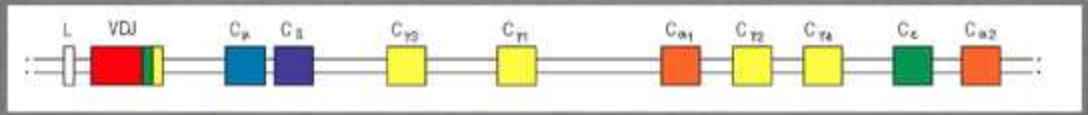


Figure 2. Distribution of Mutations in Somatic Hypermutation of Ig Genes

from Longacre and Storb Cell 102: 541, 2000.

The rearrangement of V,D, and J segments produces a functional exon that encodes the variable region of an Ig chain; together with the nearby exons encoding the constant region it constitutes the complete Ig gene.

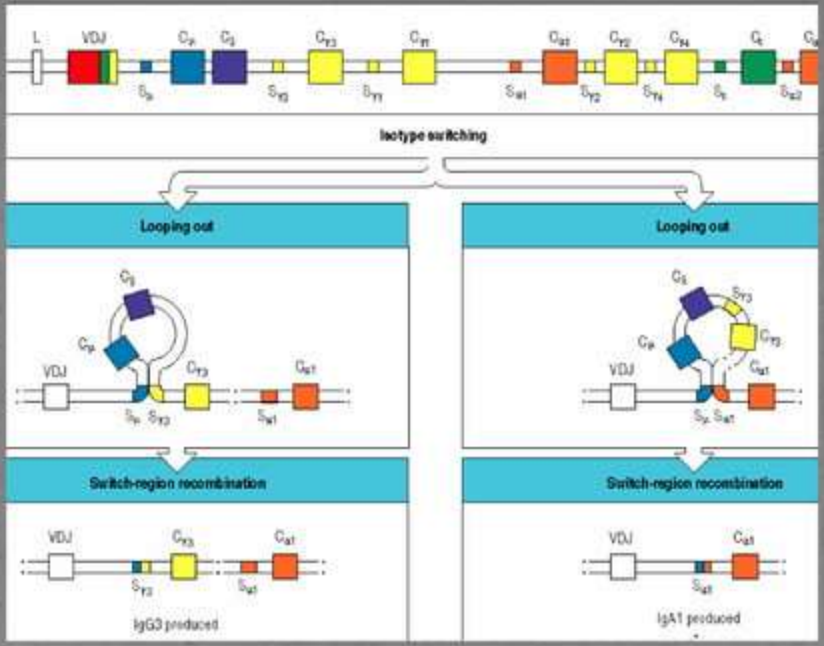


At the H chain locus, the constant region gene segments (defining the Ig classes) are arranged next to each other.

When a B cell expands into a clone, it may switch its Ig class. When this happens, the variable region of the antibody stays the same, but the constant region changes.

Isotype switching involves recombination between specific *switch regions*

- Switch regions are repetitive DNA sequences at the 5' side of each C region
- Switching occurs by recombination between switch regions, with deletion of the intervening DNA


L
VDJ
Isotype switching
Looping out
Looping out
VDJ
VDJ
Switch-region recombination
Switch-region recombination
VDJ
VDJ
IgG3 produced
IgA1 produced

Comparison of VDJ recombination, class switch recombination and somatic hypermutation

Process	Type of change	Recognition sequence	Mechanism	Factors involved
VDJ recomb.	recomb. + mutation	heptamer + nonamer	dsDNA breaks	RAG1 RAG2
Class switch	recomb.	S regions (repetitive)	dsDNA breaks	AID
Hyper-mutation	mutation	None (enhancer directs)	Deamination of Cytidine	AID

Activation-induced cytidine deaminase (AID)

- Discovered as an induced gene in a cell line with inducible class-switch recombination (subtractive hybridization)
- Transfection into B cell lines induces class switch recombination
- AID KO mice have no class switch recombination AND no somatic hypermutation
- Hyper-IgM syndrome type 2 (autosomal) is due to mutation in AID; very similar phenotype to mice (no IgG, IgA, IgE; very much reduced somatic mutation)

AID: How does it work?

- AID is closely related to ABOBEC-1, a cytidine deaminase that edits mRNA for Apolipoprotein B
- indirect action or direct action in class switch and hypermutation?

  AID could edit mRNAs for factors that act in class switch and factors that act in class switch

  OR

  it could act directly in both processes

AID as a mutator of DNA

- AID is mutagenic in bacteria; and mutations are increased by deficiency in Uracil-DNA glycosylase (enzyme that removes U from DNA and triggers DNA repair)
- Class switch is inhibited and hypermutation perturbed in UNG-deficient mice
- These results favor the hypothesis that AID directly acts on C residues in DNA to promote class switch and hypermutation

In hypermutation:

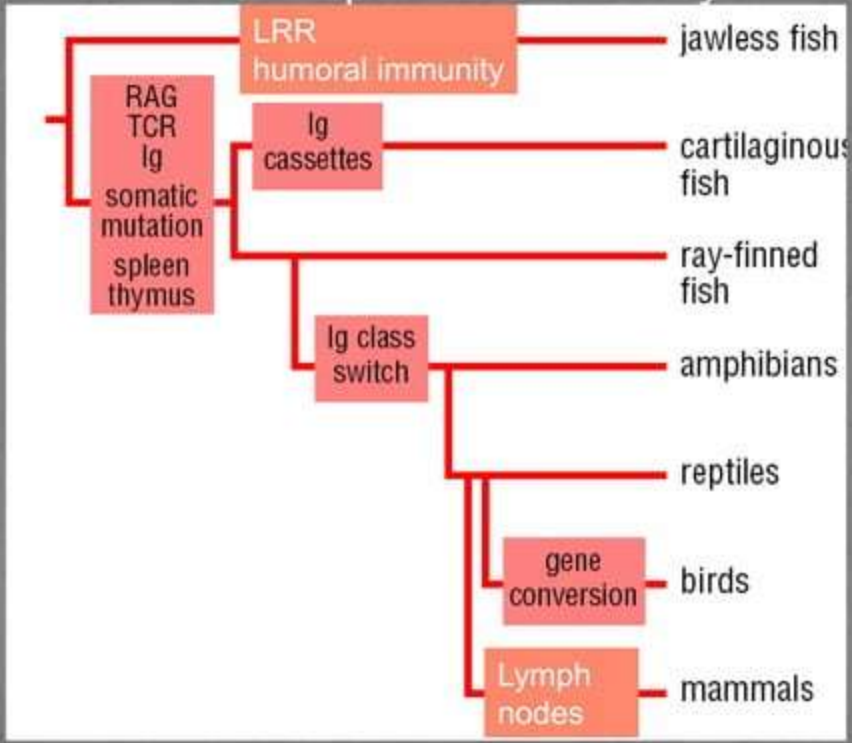
dU in DNA causes mutations through pairing with A, rather than with G, as C does;
and more mutations are introduced via mismatch repair and/or error-prone DNA polymerases.

In class switch recombination:

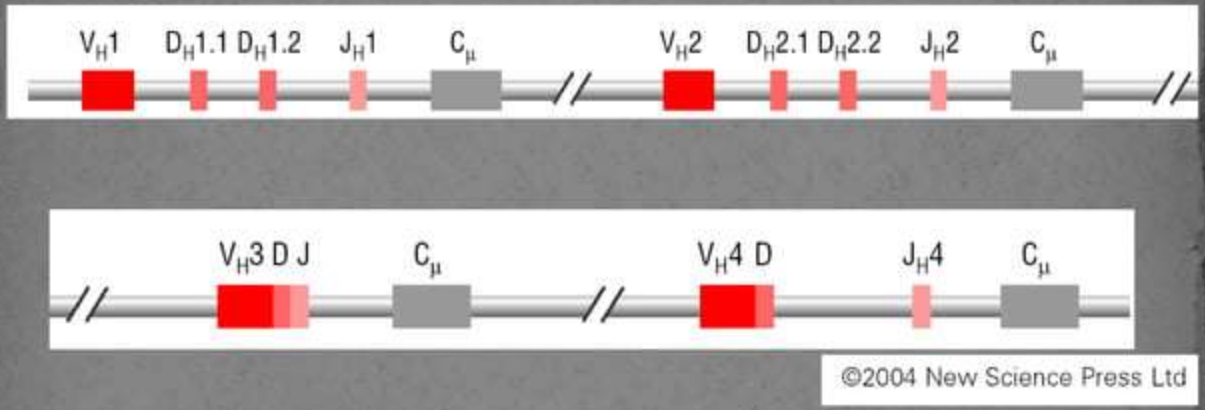
dU in DNA could lead to nick formation by repair enzymes:

nicks on both strands-->ds breaks-->recombination

Evolution of adaptive immunity

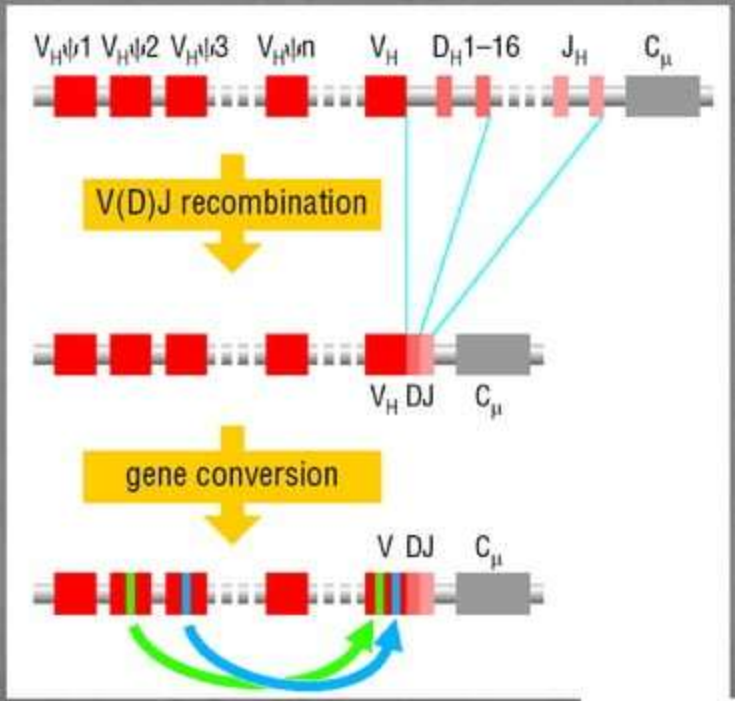


Shark Ig gene cassettes



Repeating cassettes of unrearranged and/or pre-rearranged VDJC heavy chain or VJC light chain genes. How is expression controlled?

Chickens create variability by gene conversion (AID-dependent)



To construct an immune system