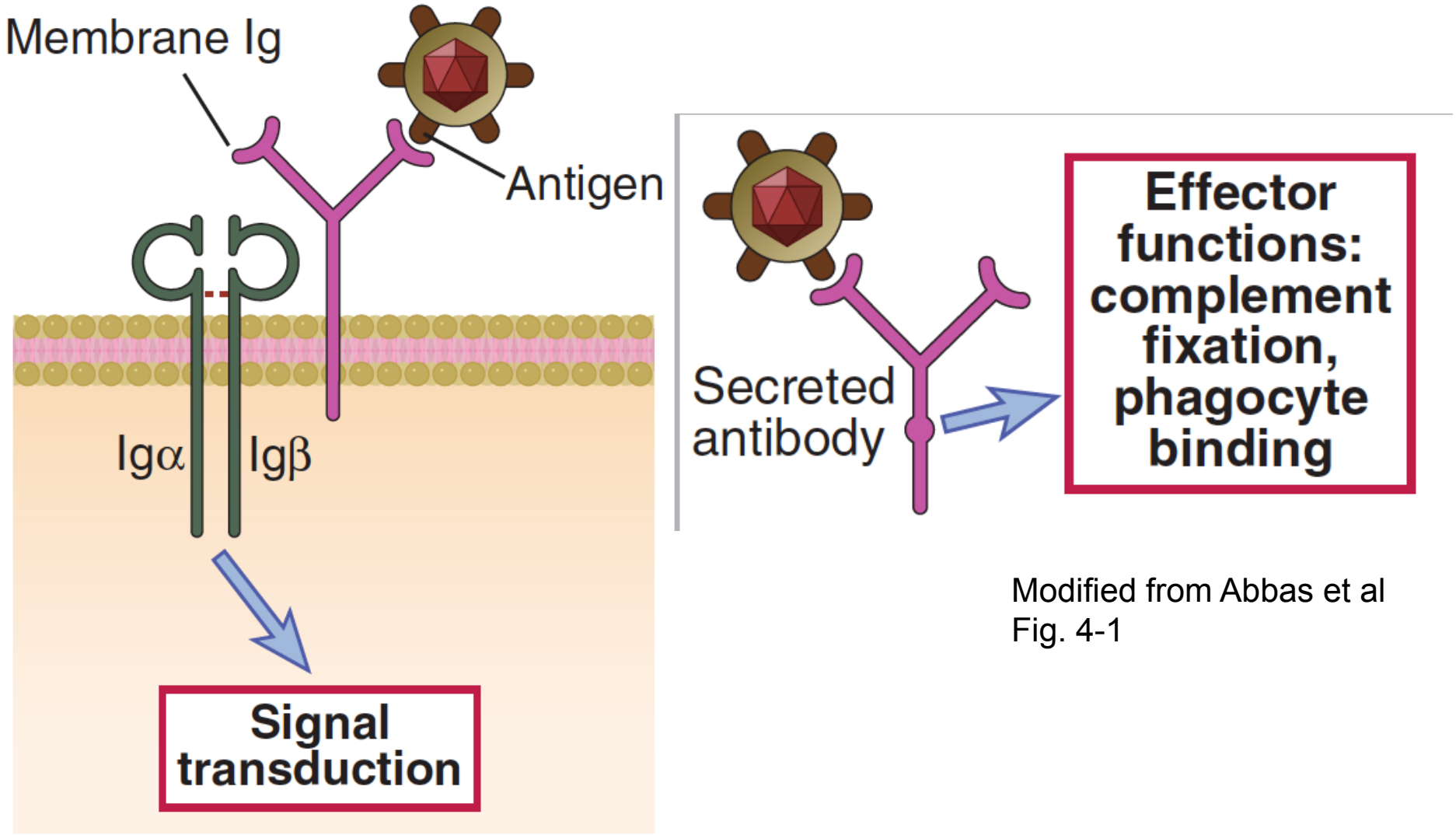
Antibodies

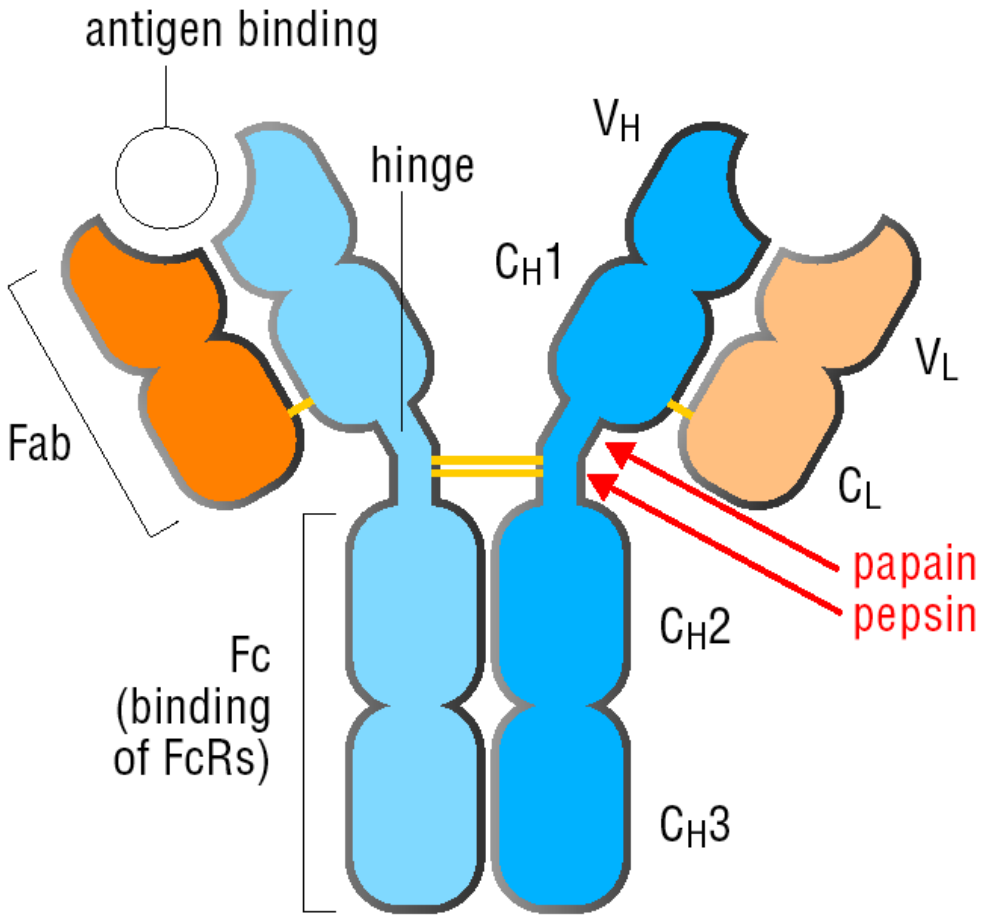
- Secreted by B lymphocytes
- Great diversity and specificity: $>10^9$ different antibodies; can distinguish between very similar molecules
- Tag particles for clearance/destruction
- Protect against re-infection (vaccines)

Antibodies: antigen receptors→ clonal selection→ secreted antibodies



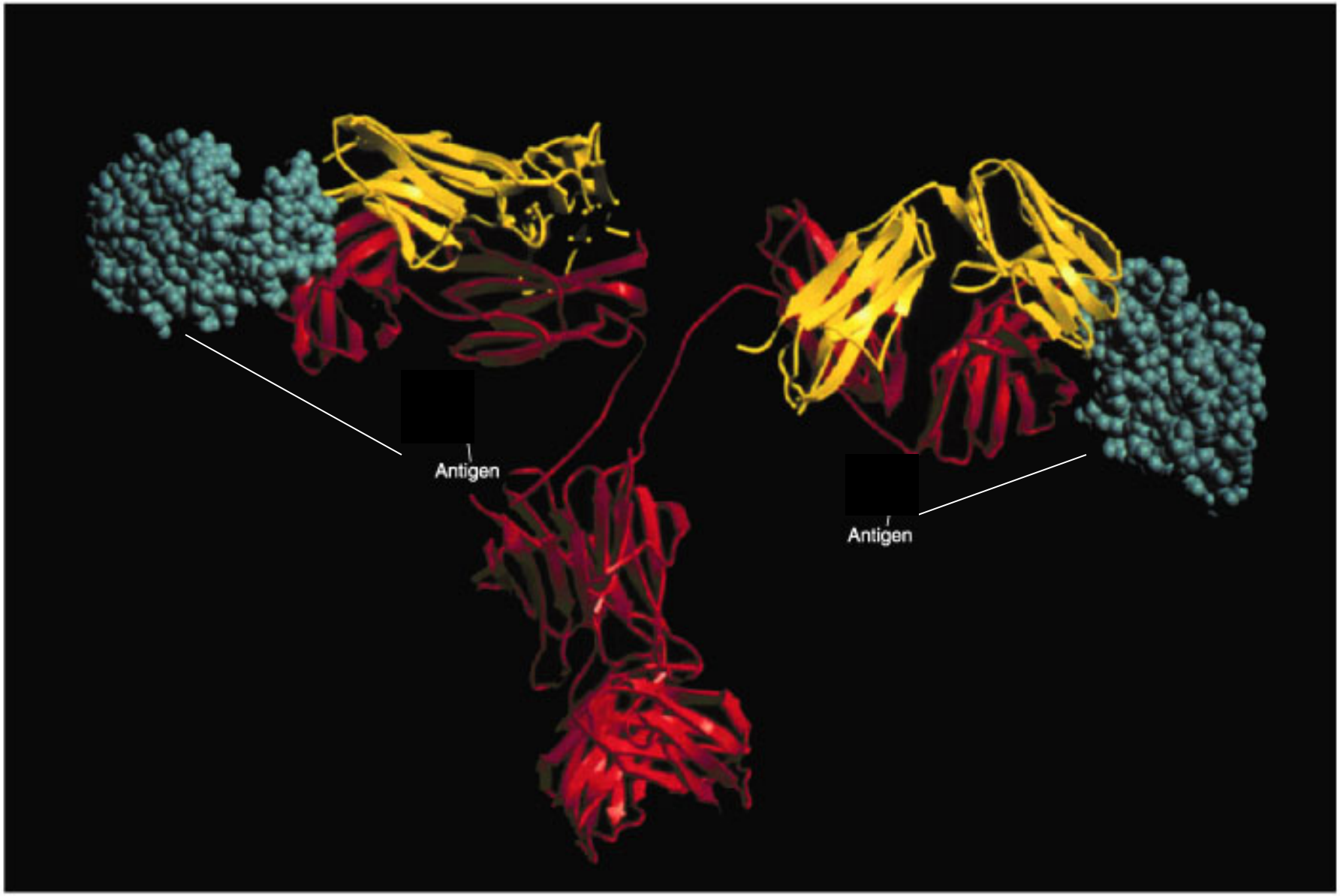
Modified from Abbas et al
Fig. 4-1

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)

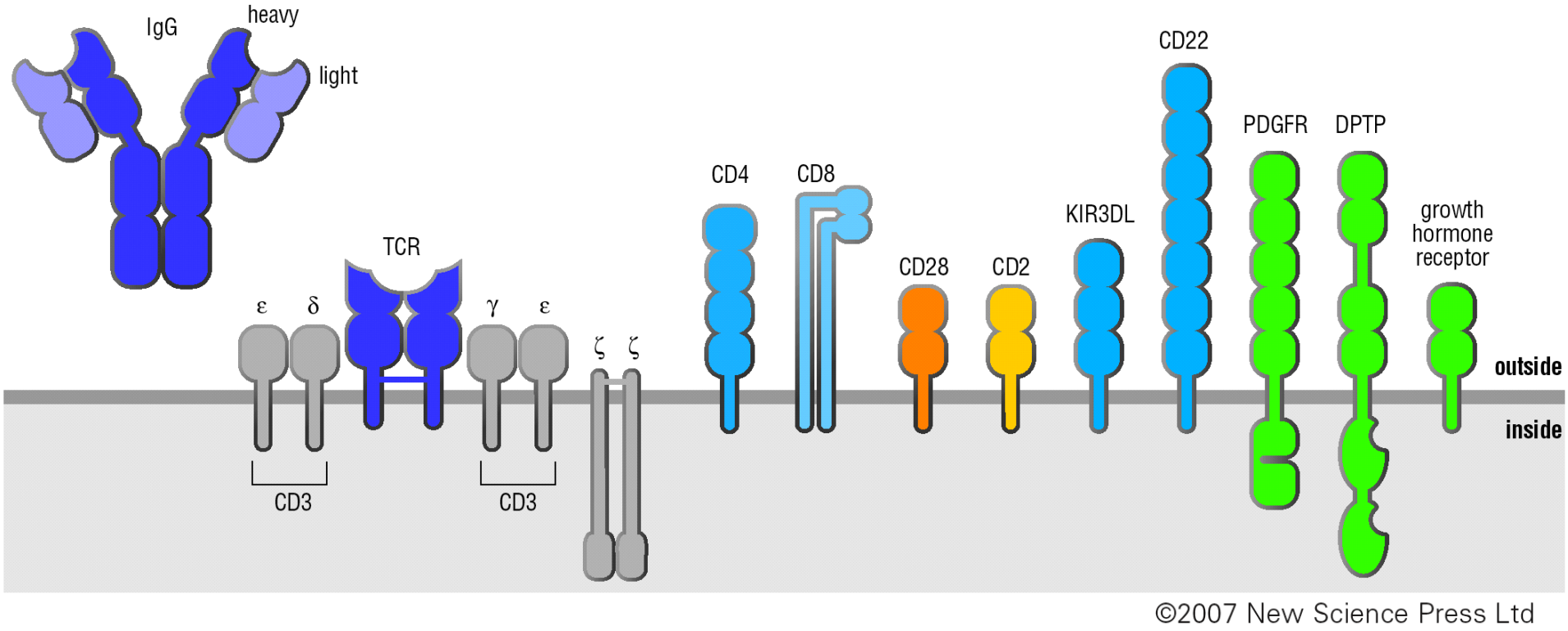
Binding of an antigen by an antibody.



Antibody Structure supports Function

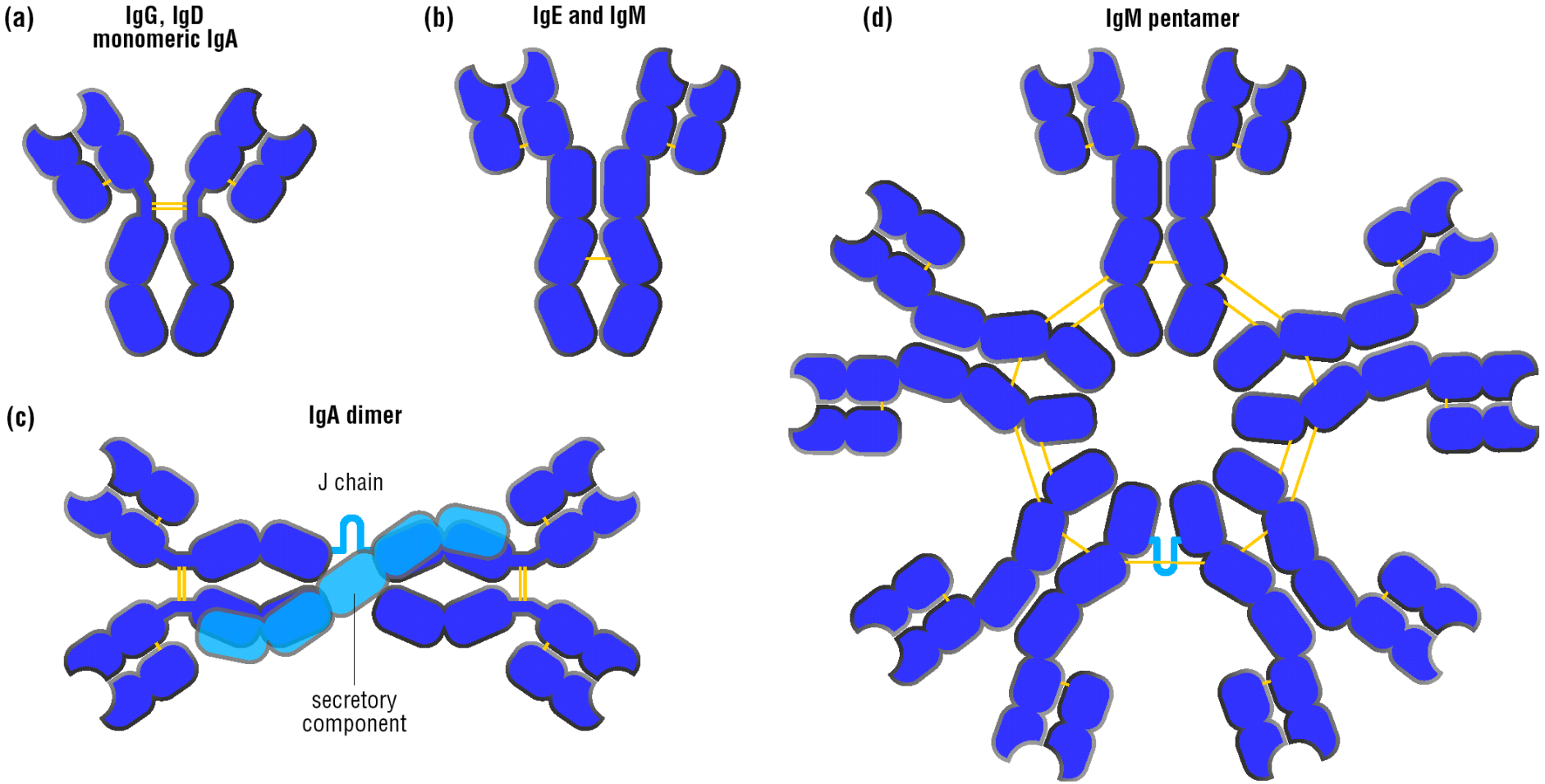
Variability in antibodies is clustered in the loops in the Variable domains of the heavy and light chains (green);
Green areas are called: hypervariable regions or complementarity determining-regions

The Immunoglobulin Superfamily (a few examples)



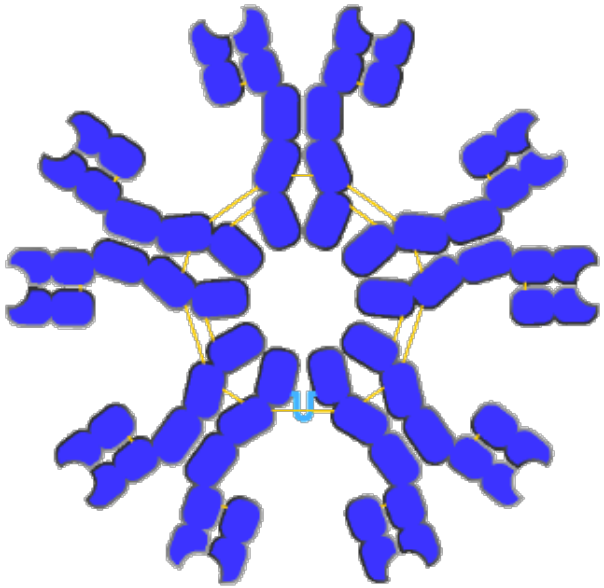
Ig and TCR are very similar structurally

Antibody Classes: Structure



Antibody Classes: Structure supports function

- IgM: 1st type made
  - Antigen receptor of naïve B cells
  - Secreted version has 5 units stuck together in "pentamer"
  - IgM is first antibody produced in immune response: multimeric structure adds to "avidity" for antigen

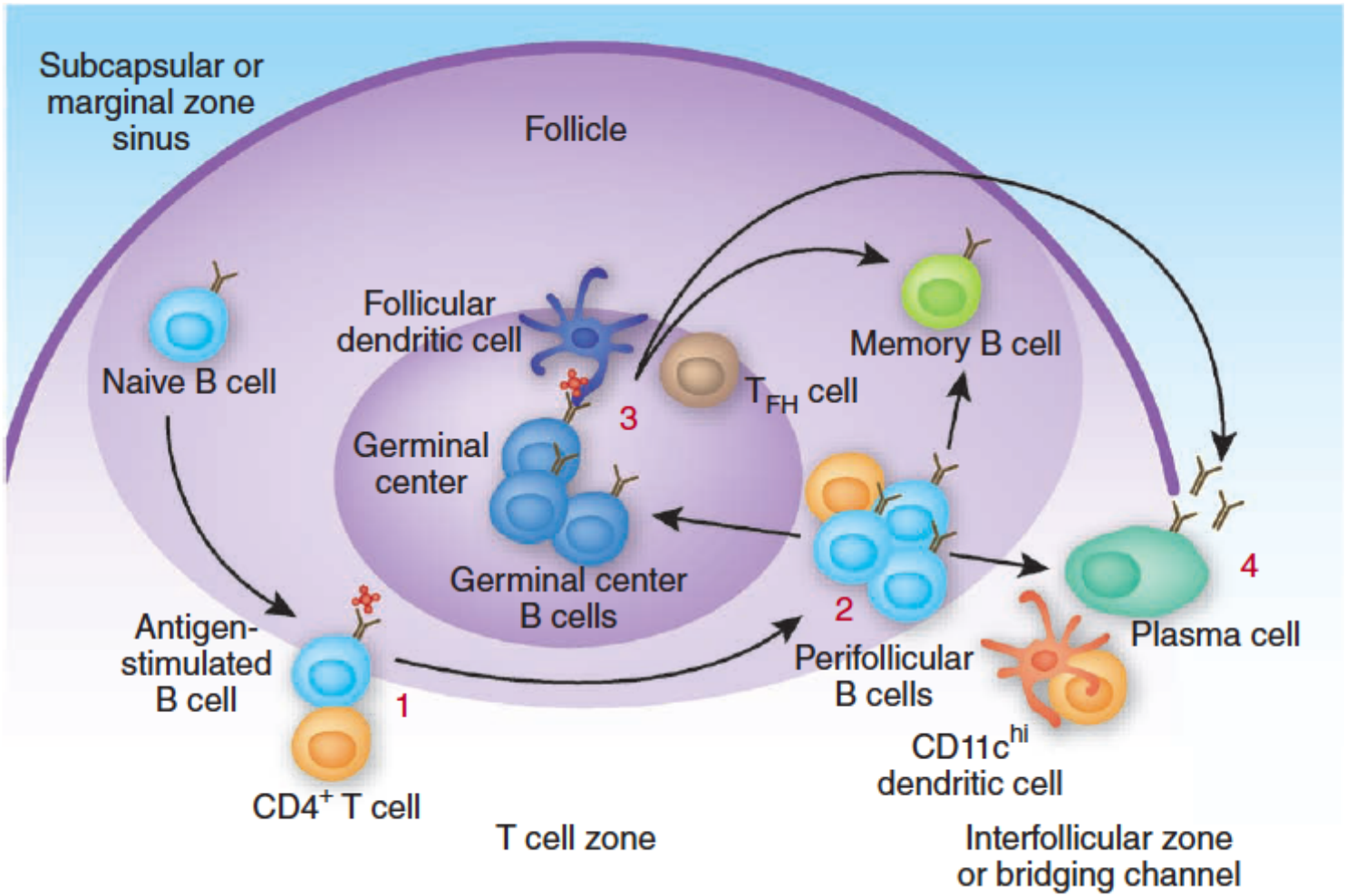


Affinity and Avidity

Affinity: the strength of binding between a single binding site and a single ligand $K_D = \frac{[A][B]}{[AB]}$

•**Avidity**: the strength of multivalent binding between a molecule, particle, or cell and a complex ligand.

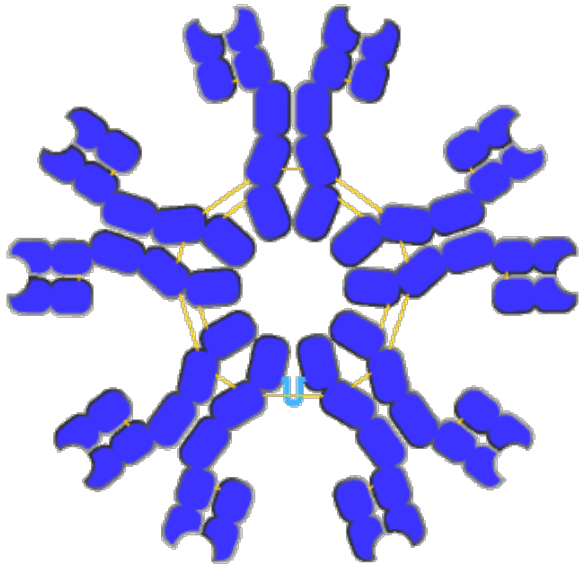
Diversification of Antibodies during immune responses: affinity maturation and class switch recombination



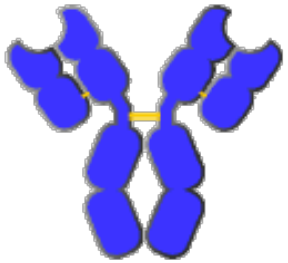
Goodnow et al, Nature Immunol. 2010

Affinity and Avidity II

- IgM is produced early in an immune response when the affinity for antigen often is low (avidity comes from multimerization);
- IgG, IgE and/or IgA are produced later in an immune response when antibody affinity has improved
  - Avidity is maintained by higher affinity, despite fewer binding sites
  - Diversification of functions with different classes

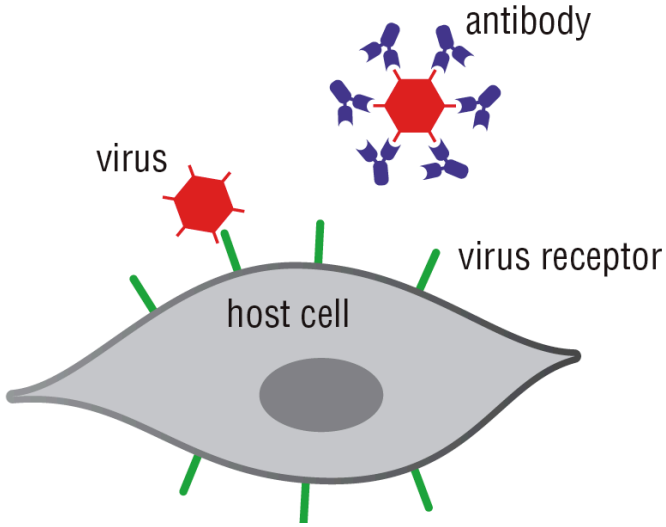


IgM

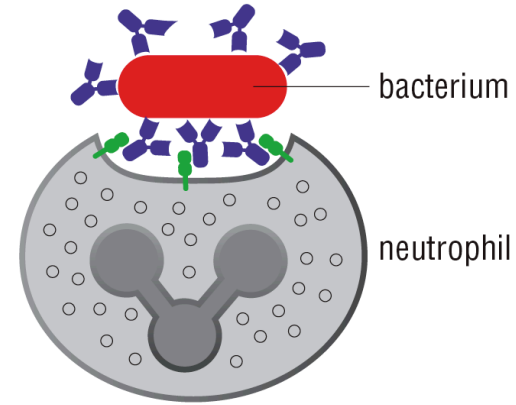


IgG

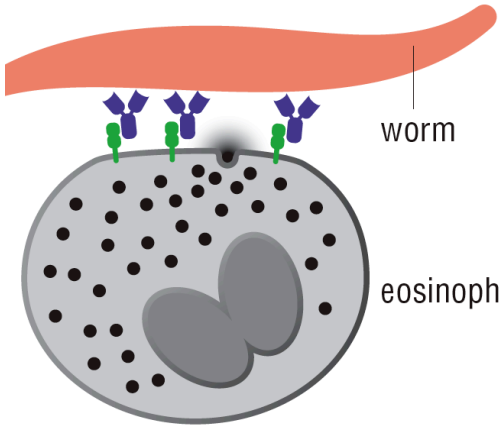
Antibodies can be directly protective or can promote immune protective mechanisms via other cells or molecules



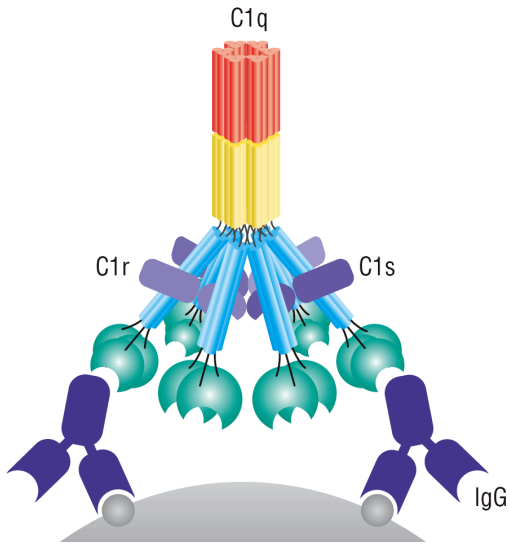
neutralization



phagocytosis



ADCC



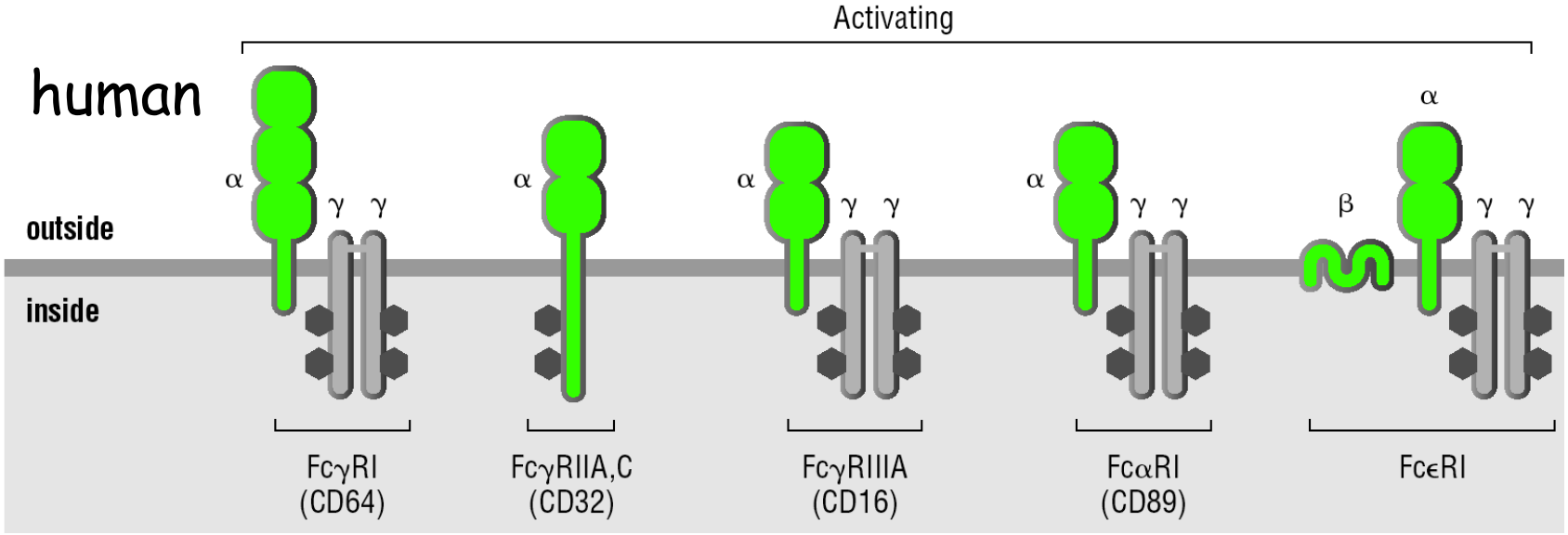
activation of complement

From **Immunity: The Immune Response in Infectious and Inflammatory Disease**
by DeFranco, Locksley and Robertson

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

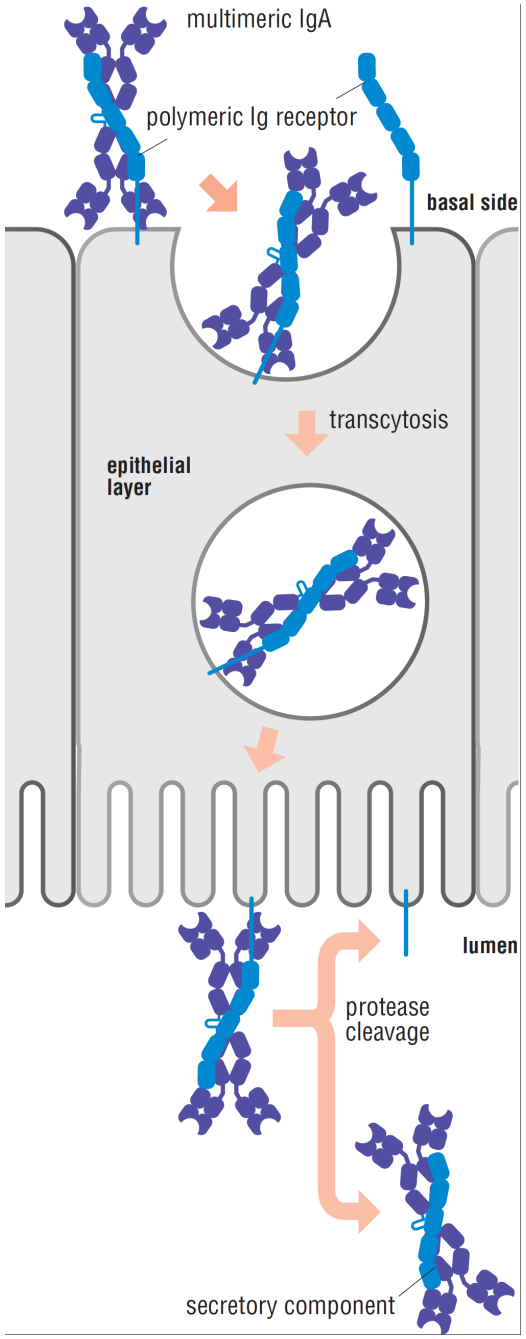
Fc receptors bind particular antibody isotypes and contribute to effector functions of antibodies



- There are a number of activating FcRs, which utilize ITAM signaling like BCR, TCR, some innate receptors (the two hexagon motif represents the ITAM)
- There is also an inhibitory FcR (IIB), which has an ITIM that inhibits ITAM signaling
- Effector functions: phagocytosis, degranulation, antibody-dependent cytotoxicity

Fc receptors retain IgG in the blood and transport IgA (IgM) across epithelial barriers

- IgA is secreted in mucosal tissue and is transported across mucosal epithelial barriers by the poly-Ig receptor
- Poly-Ig receptor is cleaved; the part that stays bound to IgA is “secretory component”; protects from proteases
- FcRn (neonatal FcR) transports IgG across placenta (maternal to fetus) and it preserves IgG in the blood (prevents clearance/catabolism)

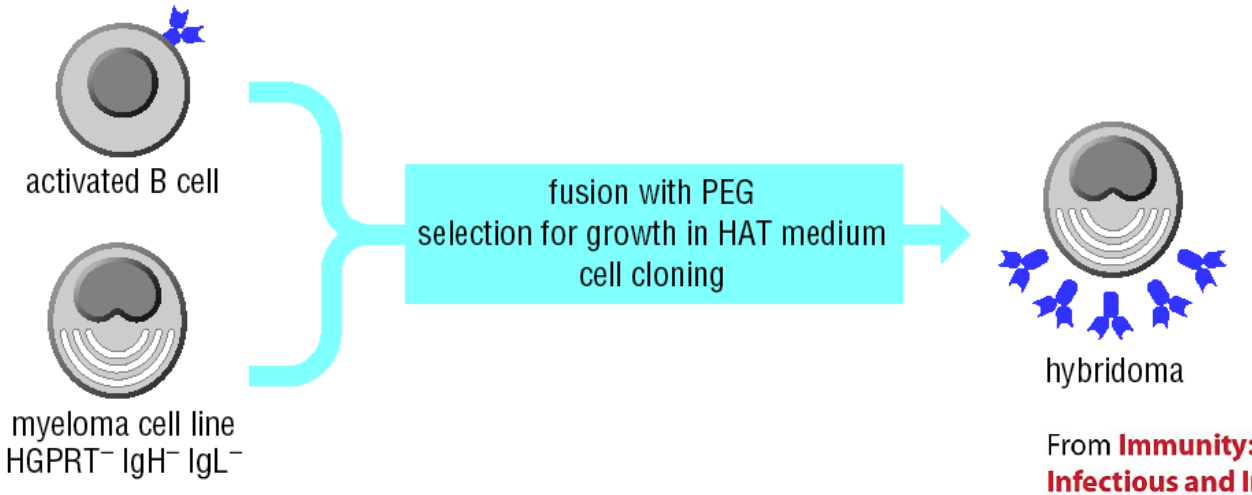


Antibodies and medicine

- Vaccines: mostly work by protective antibodies
- Antibodies and diagnosis
- Antibodies can provide “passive immunity” (tetanus, snake bites, intravenous immune globulin=“IVIG”)
- Monoclonal Antibodies as therapeutics: (cancer, autoimmune disease, etc.): several new ones approved each year currently

Monoclonal Antibodies

- Normal antibodies are "polyclonal"; mixtures of antibodies made by several different clones of B cells
- Monoclonal antibodies: Single antibody (all same H and L chains): more reliable, consistent; can be produced in unlimited quantities
- Most common method of creation: fuse together B cells and a myeloma cell line ("hybridoma")



From **Immunity: The Immune Response in Infectious and Inflammatory Disease**
by DeFranco, Locksley and Robertson

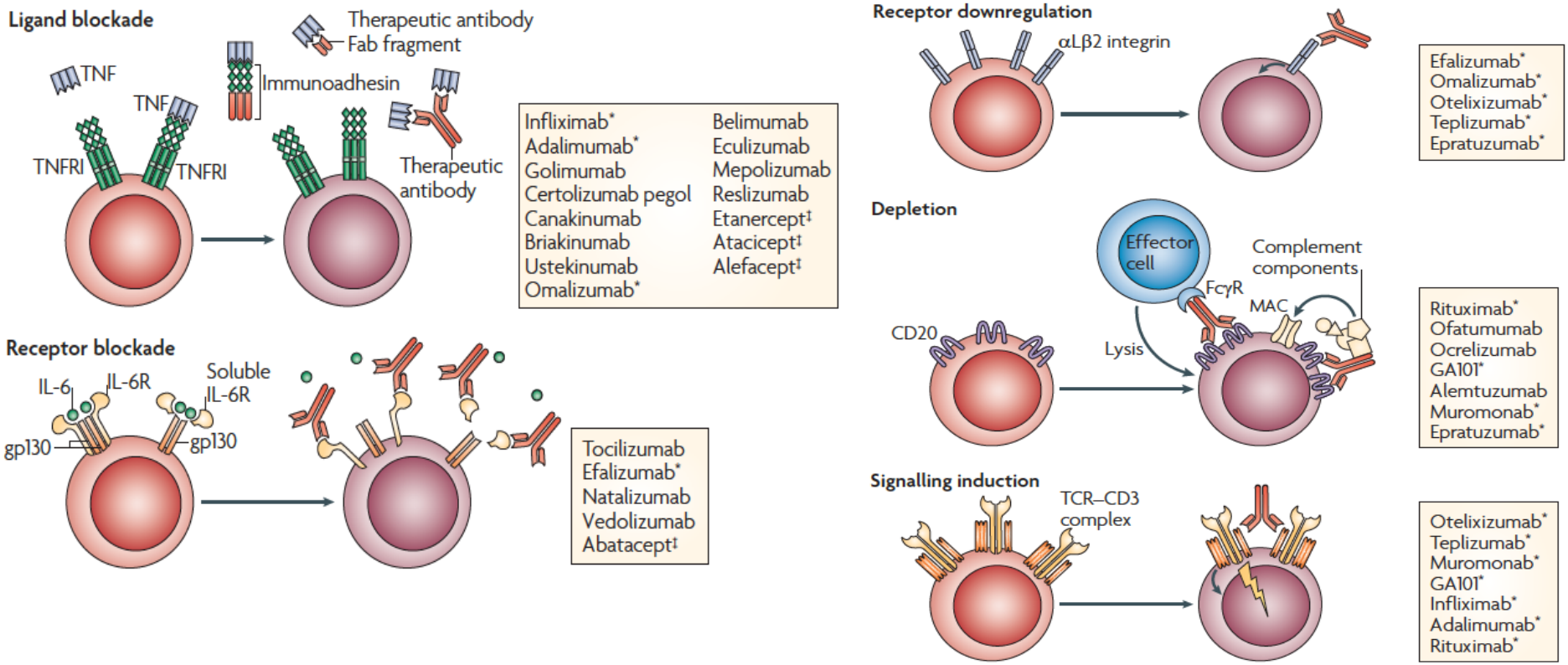
Monoclonal antibodies used in medicine

Standardized, unlimited 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

Monoclonal antibodies used in medicine



Chan and Carter Nat. Rev. Immunol. 10:301, 2010

Monoclonal antibodies used in medicine

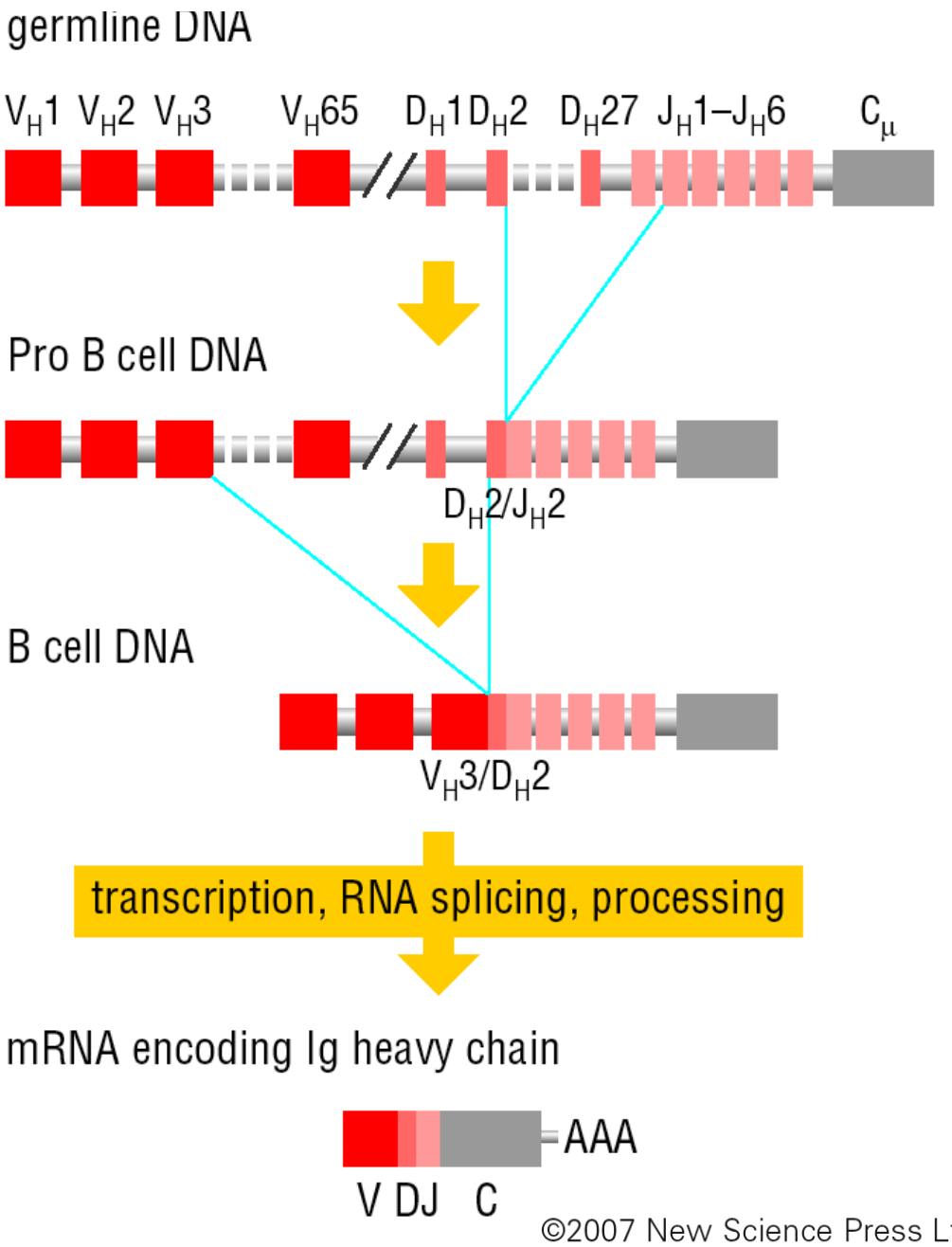
Variations of antibodies are becoming novel therapeutics:

- Antibody-drug or Antibody-toxin conjugates
- Bispecific antibodies
- Chimeric antigen receptor (CAR) T cells

Generation of antibody diversity

- How do we make >10^9 different antibodies?
- Genes for antibodies are present in pieces that can be combined in many different combinations in different lymphocytes
  - V region of Ig L and H chains are constructed from 2 and 3 different pieces each having multiple copies

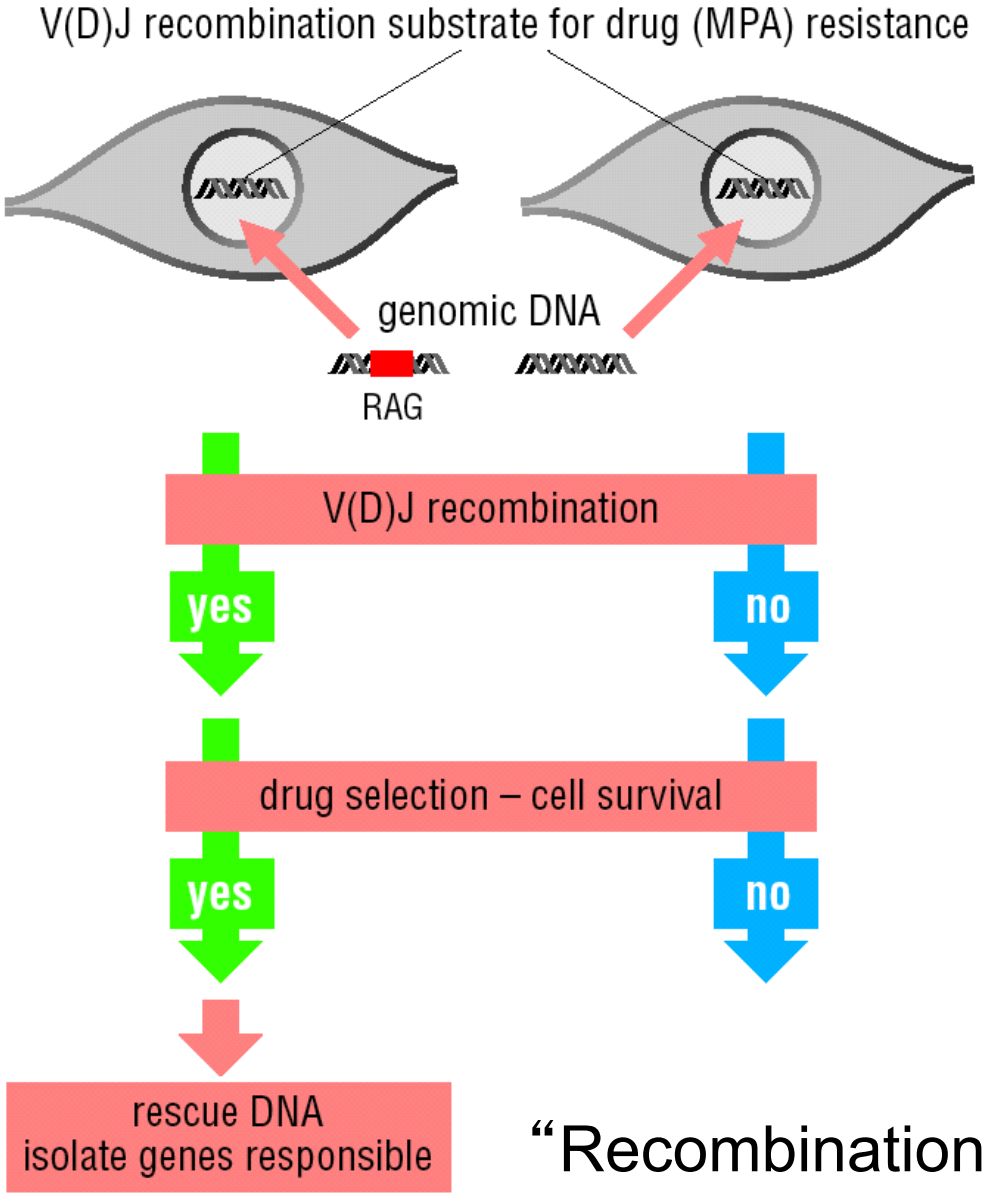
V(D)J recombination

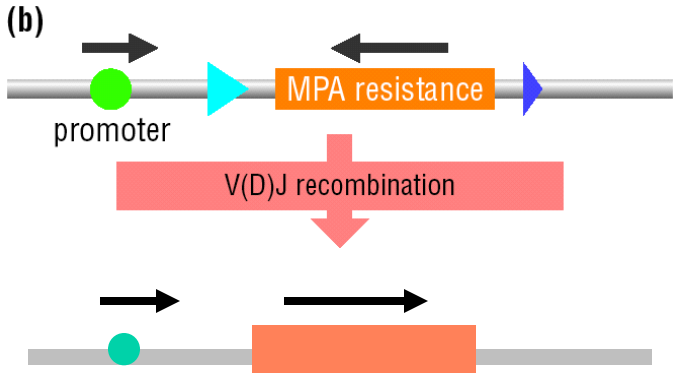


Generation of Antibody Diversity
(human numbers)

- 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 ("Combinatorial diversity")
- Also there is "Junctional diversity" (addition or deletion of nucleotides at recombination sites, especially of H chain), estimated to add substantially to overall diversity

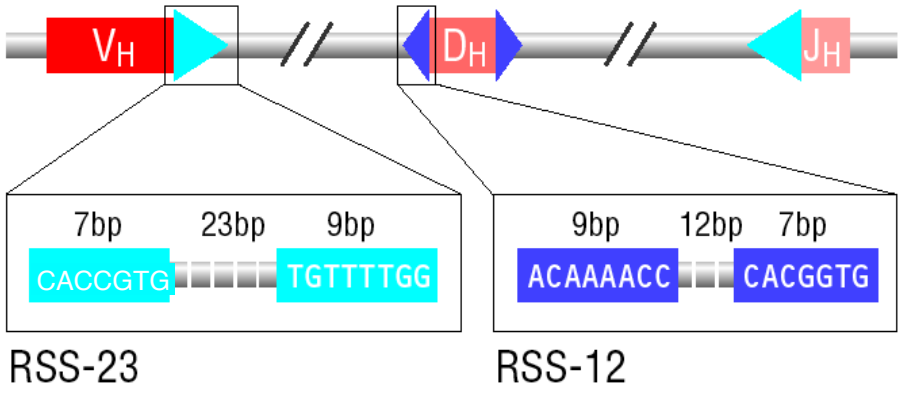
Discovery of Rag1, 2 genes

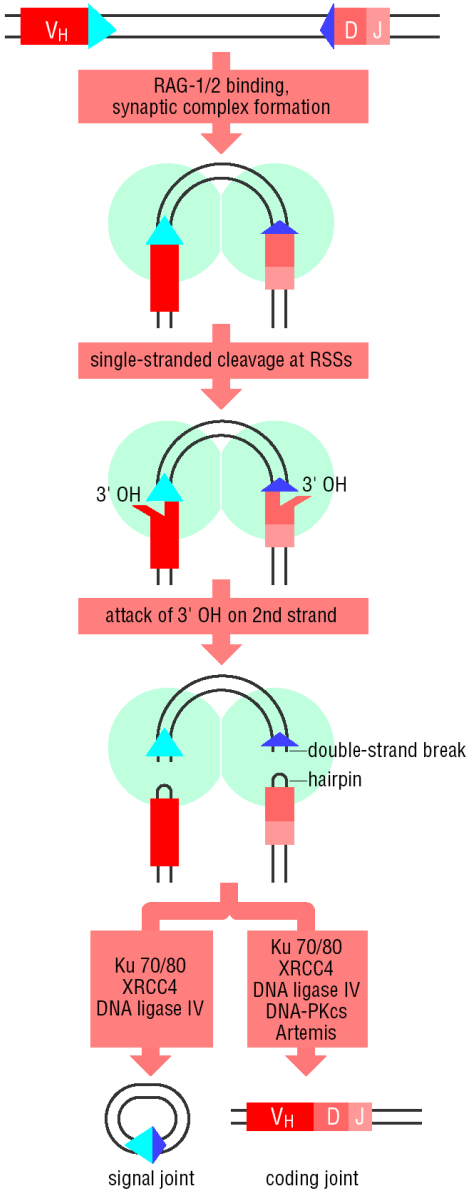




"Recombination Activating Gene"

Mechanism of V(D)J recombination





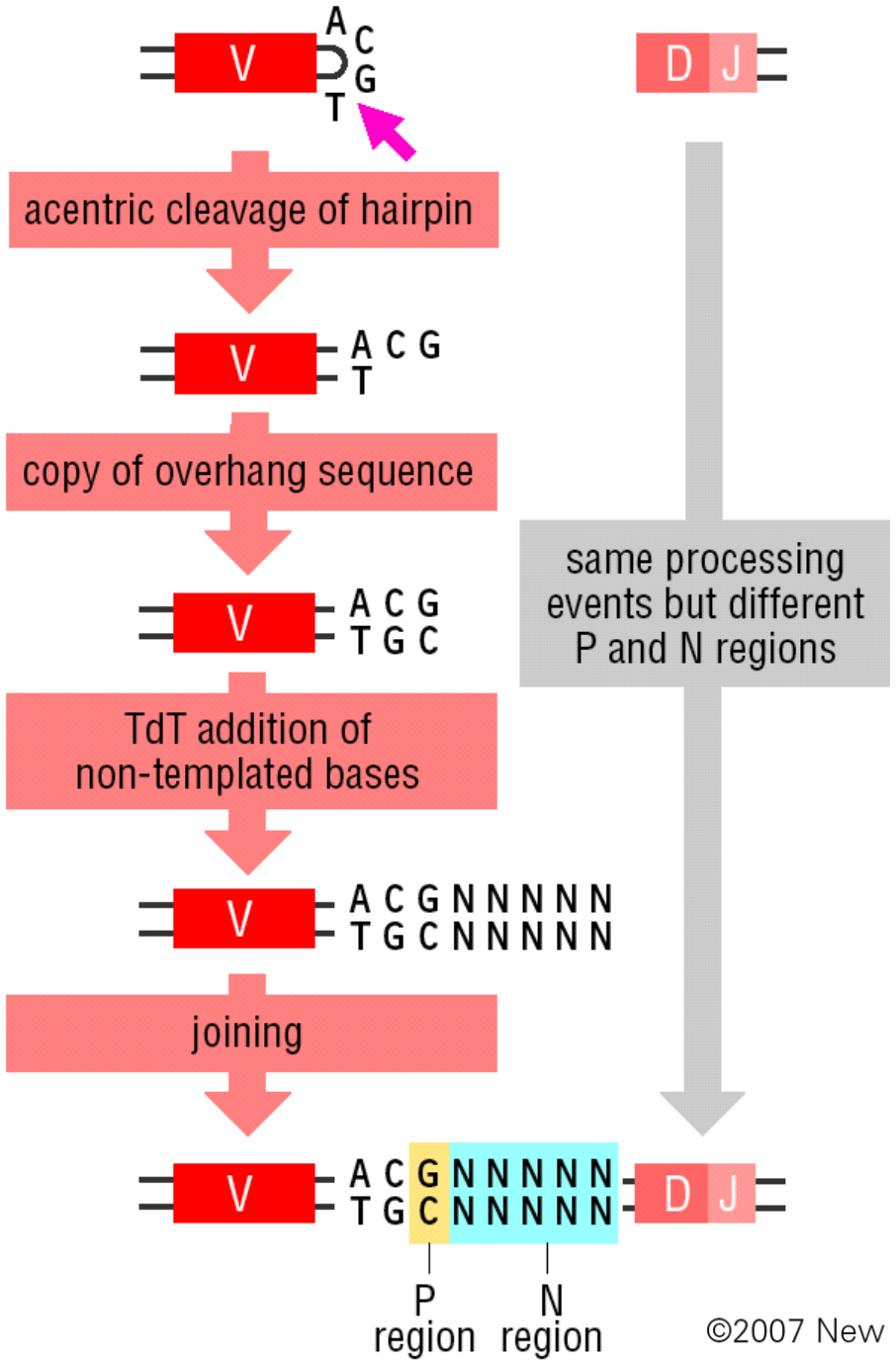
- Recombination signals (12/23 rule)
- Rag-1/Rag-2/Artemis
- DNA repair proteins
  (non-homologous end joining proteins)

- Defects: Severe Combined
  Immunodeficiency (SCID)

Creation of Junctional Diversity by P-regions and TdT

(There can also be trimming back by nucleases before addition of N regions)

Key point: reading frame must be preserved to get functional gene

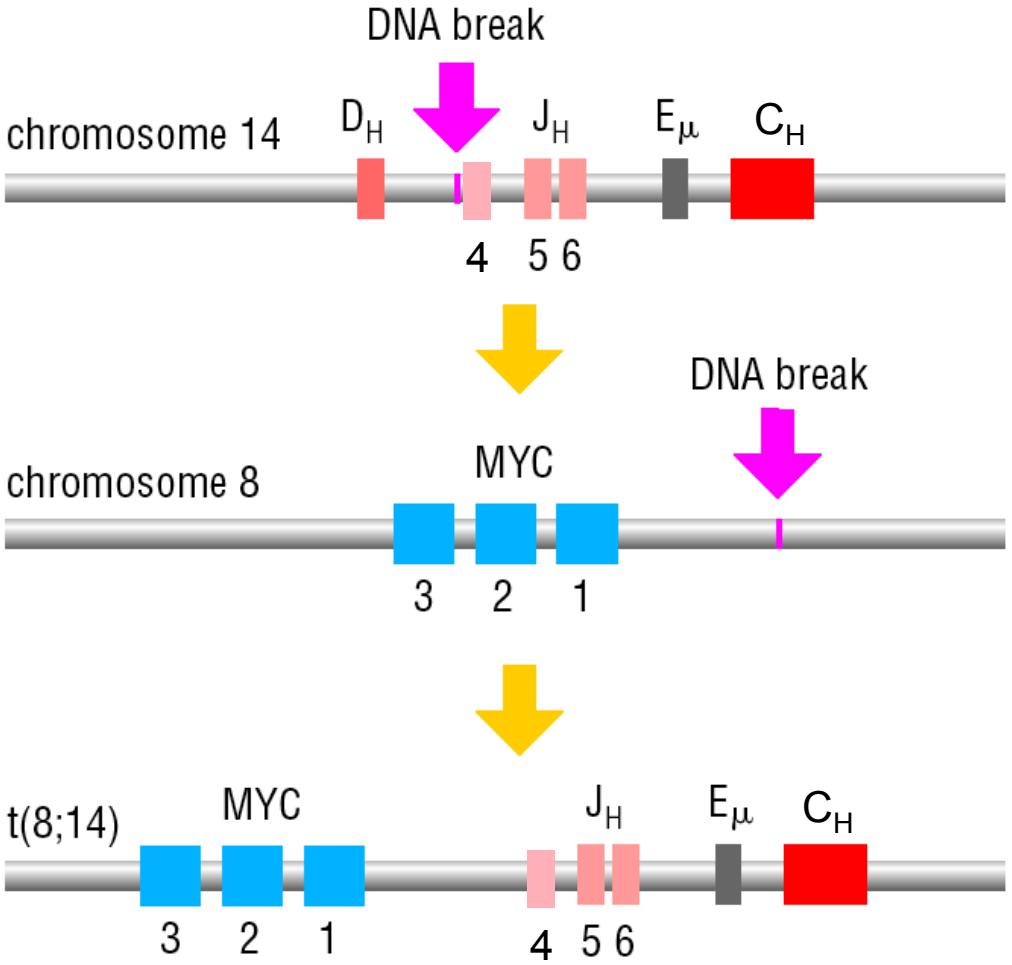


Defects in Lymphocyte development leading to severe combined immunodeficiency (SCID)

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

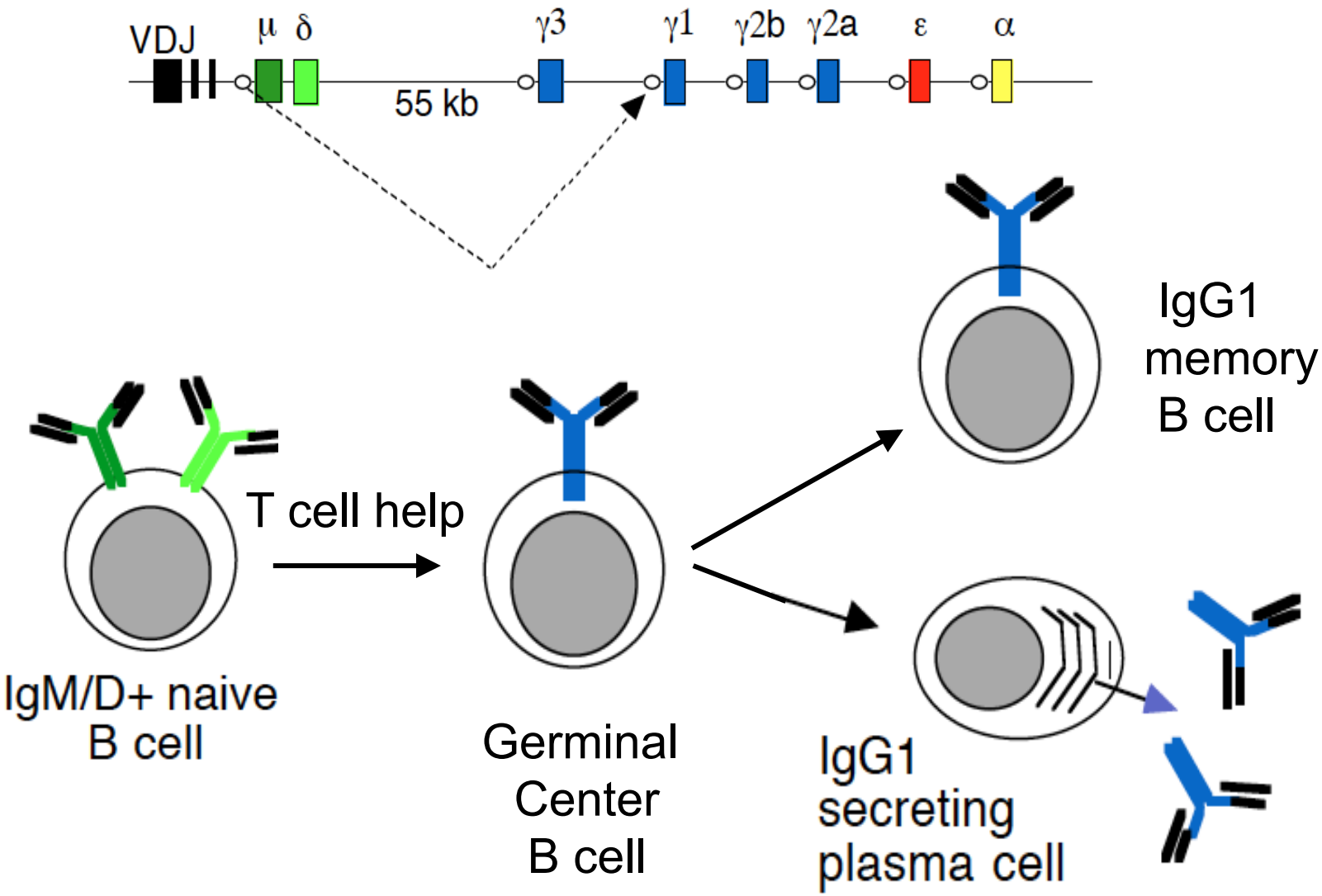
Note: SCID can also result from defects that interfere with lymphocyte development (adenosine deaminase deficiency, purine nucleotide phosphorylase deficiency, MHC defects, etc.)

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

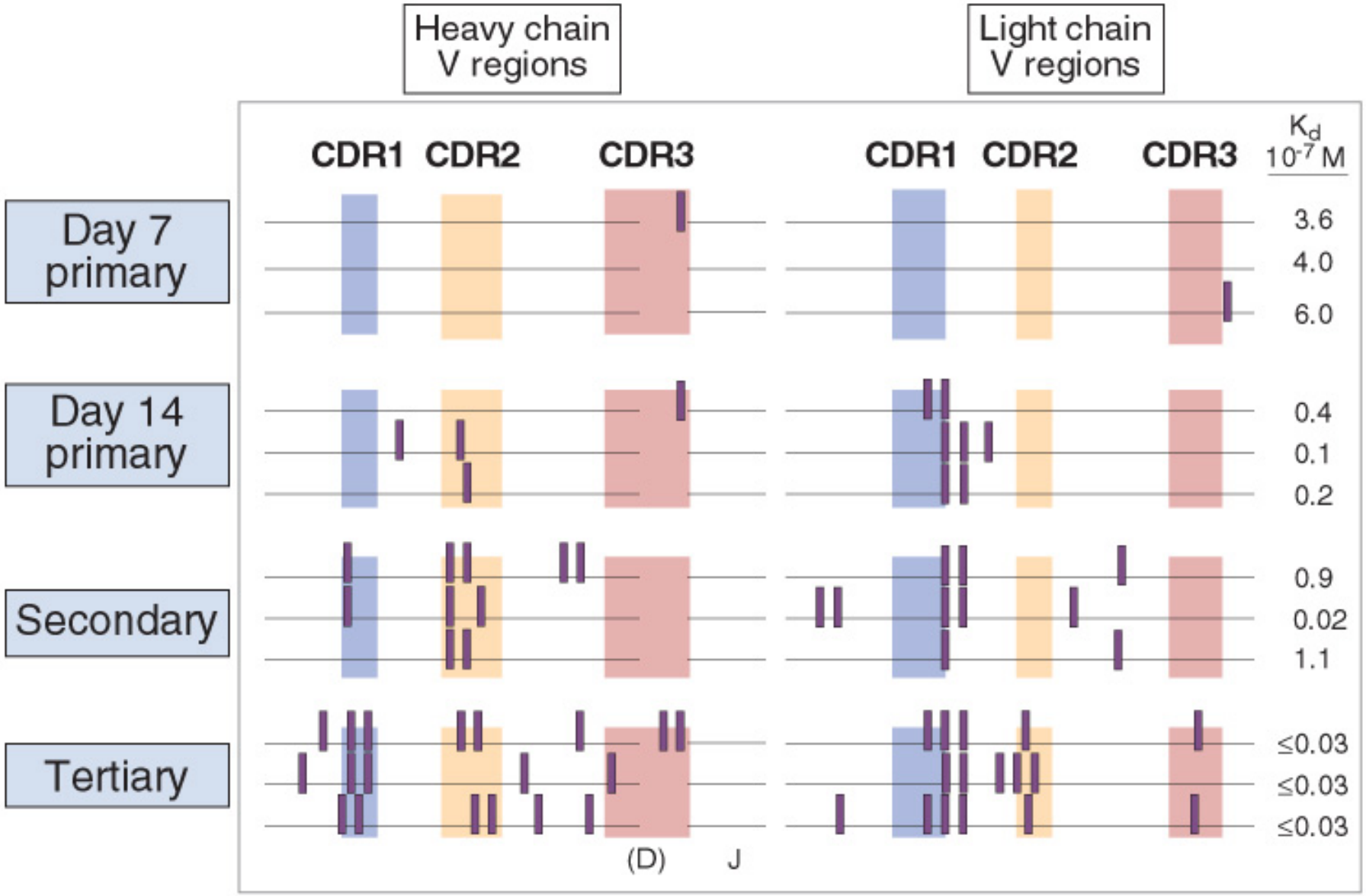


VDJ Recombination reactions contributes to translocation leading to over-expression of a cellular growth or survival promoting gene

Ig Heavy chain class (isotype) switching



Affinity maturation and antibody responses



Ig mutations are localized near transcription start site

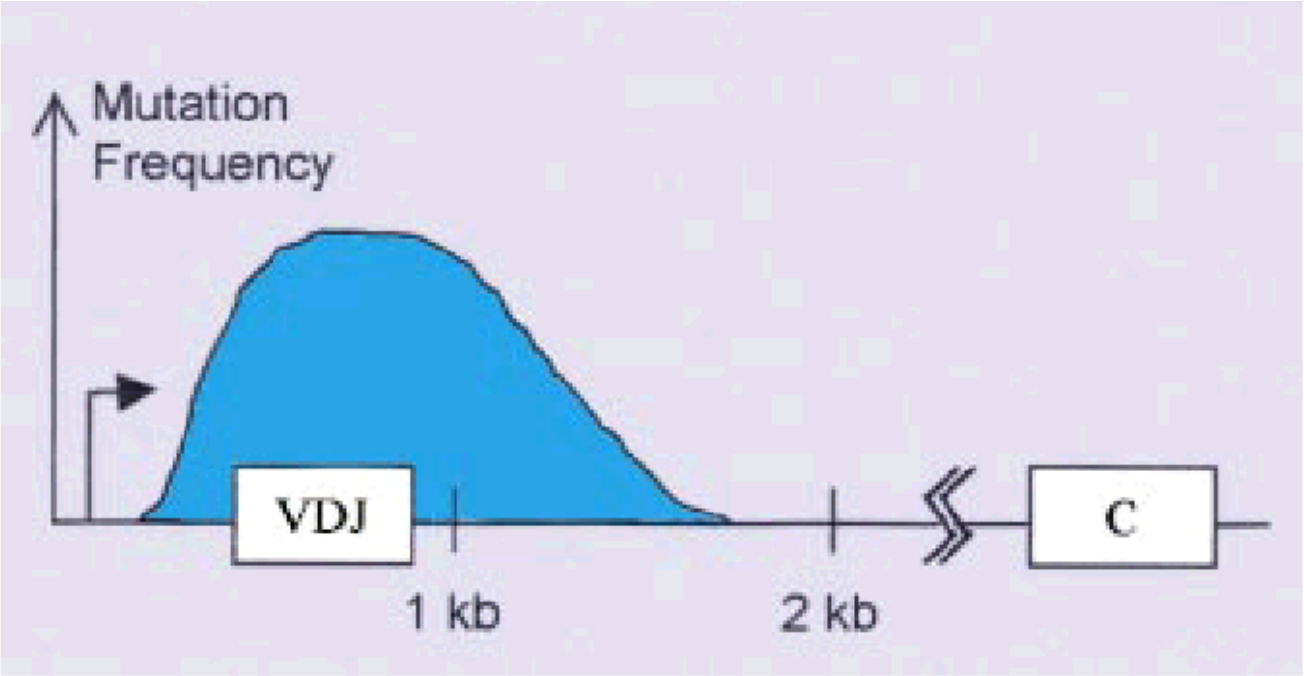


Figure 2. Distribution of Mutations in Somatic Hypermutation of Ig Genes

from Longacre and Storb Cell 102: 541, 2000.

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	
Hyper-mutation	mutation	RGYW (enhancer directs)	ssDNA nicks?	

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	RGYW (enhancer directs)	ssDNA nicks?	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 strong defect in class switch recombination AND in 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 highly related to APOBEC-1, a cytidine deaminase that edits mRNA for Apolipoprotein B (via a targeting subunit)
- 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

Model for direct actions of AID in somatic mutation and class switch

In hypermutation:

U in DNA could lead to direct mutations and secondary mutations via mismatch repair and/or error-prone DNA polymerases

In class switch recombination:

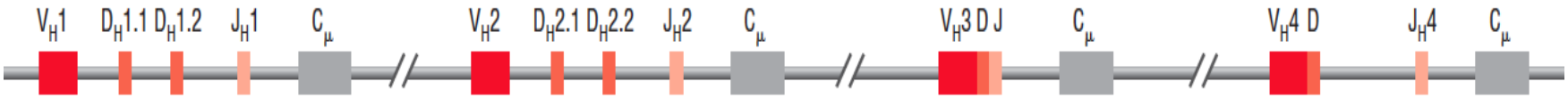
U in DNA could lead to nick formation by repair enzymes:

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

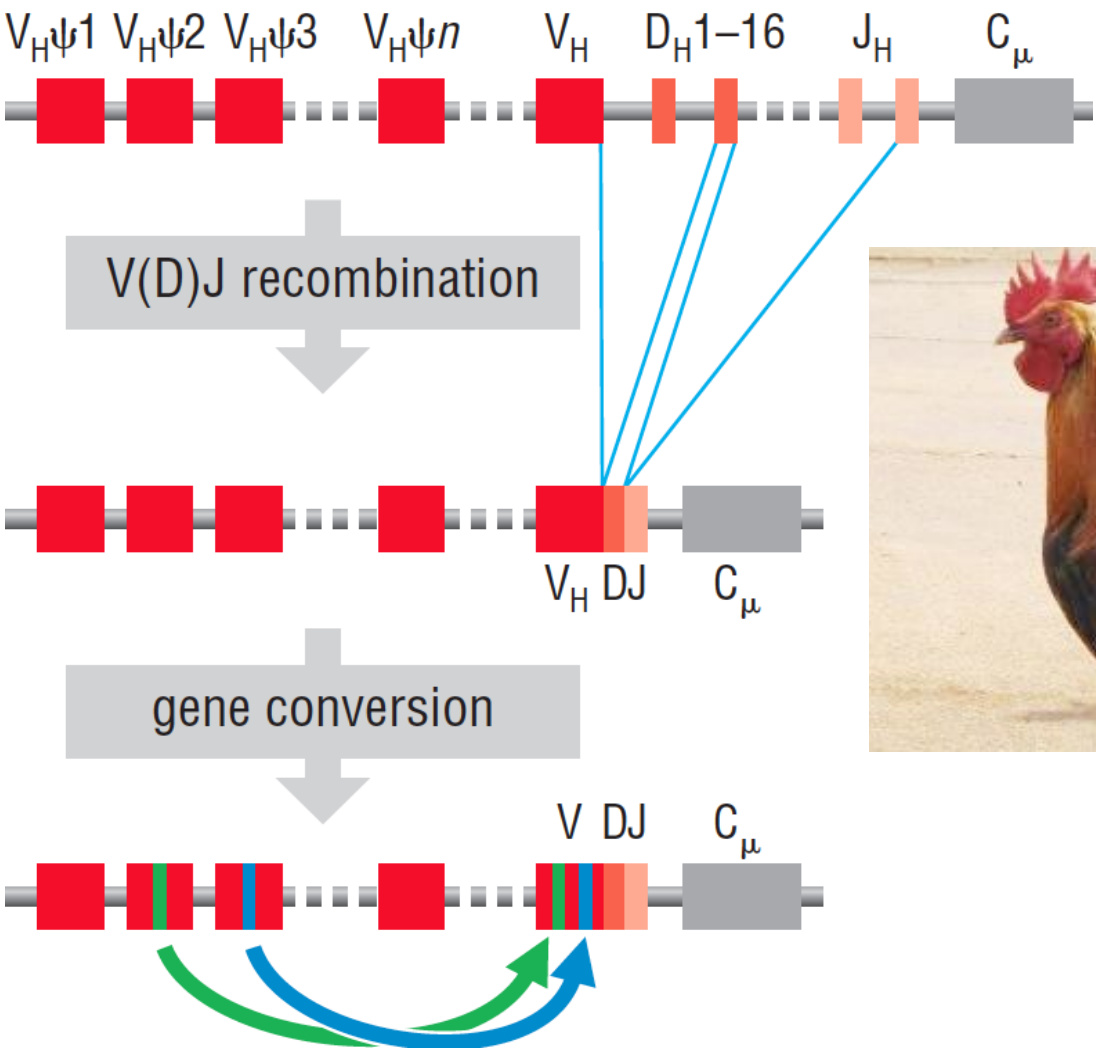
Mice and Humans have quite similar antibody genes and proteins

But other vertebrates can have striking differences

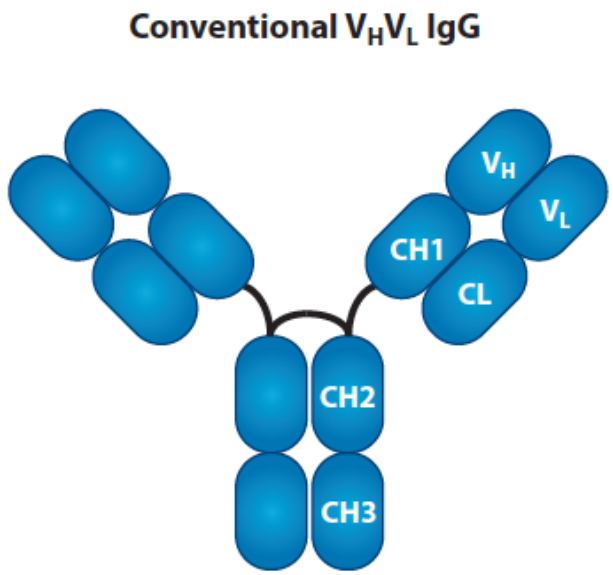
Sharks have tandemly repeating IgH cassettes in their genome

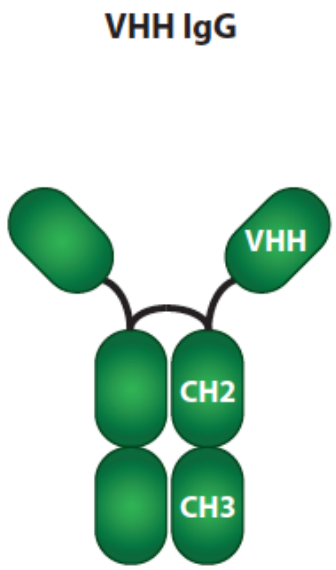


Chickens mostly diversify by gene conversion, using AID



Camelids make heavy-chain only antibodies as well as conventional antibodies

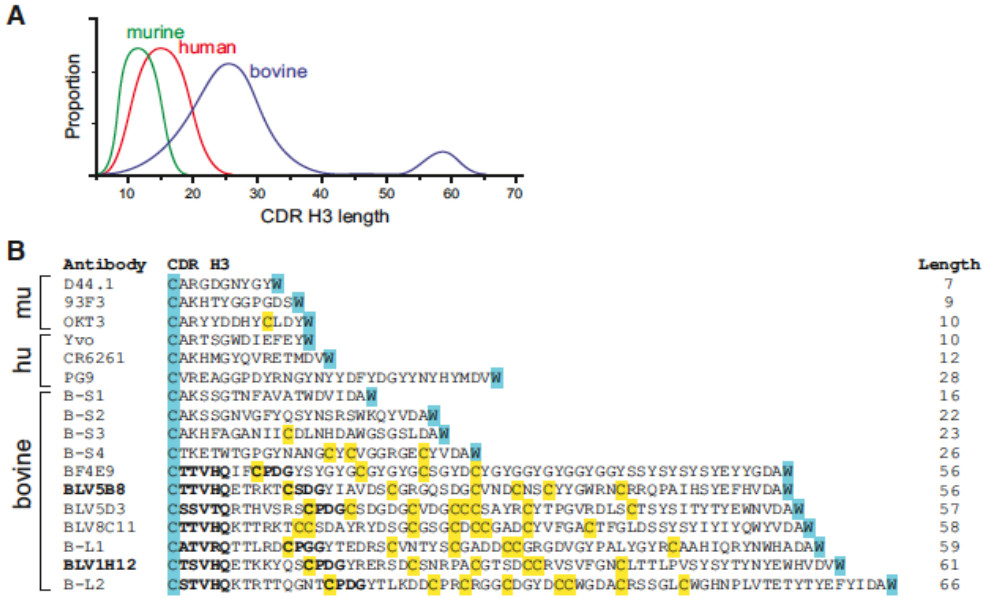


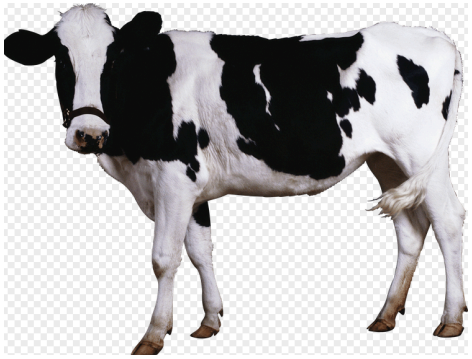


Vh only from these Ab: “nanobodies”

Ingram, Schmidt and Plough, Ann. Rev. Immunol. 36: 695, 2018

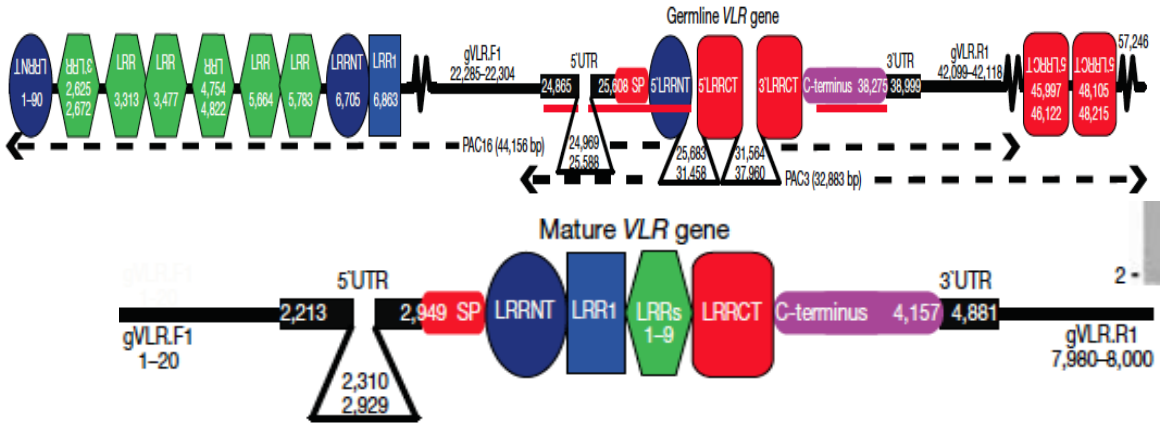
Cows make some antibodies with ultralong heavy chain CDR3 loops

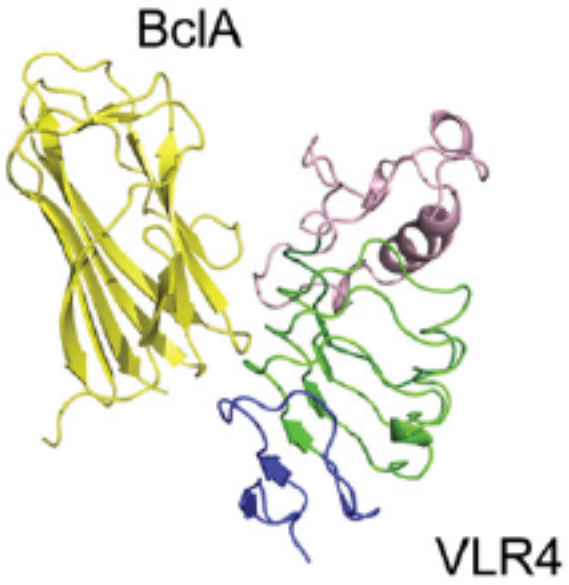




Wang, et al.
Cell 153: 1379, 2013

Lamprey and Hagfish have evolved Ig and TCR with a totally different structure





Pancer et al. Nature 430: 174, 2004
Kirchdoerfer et al. Structure 20: 479, 2012