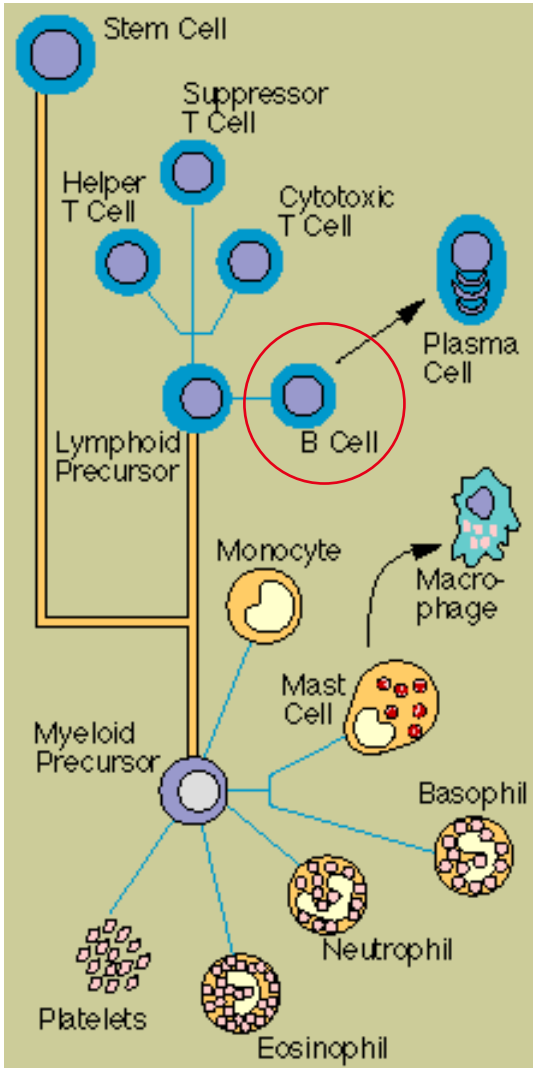
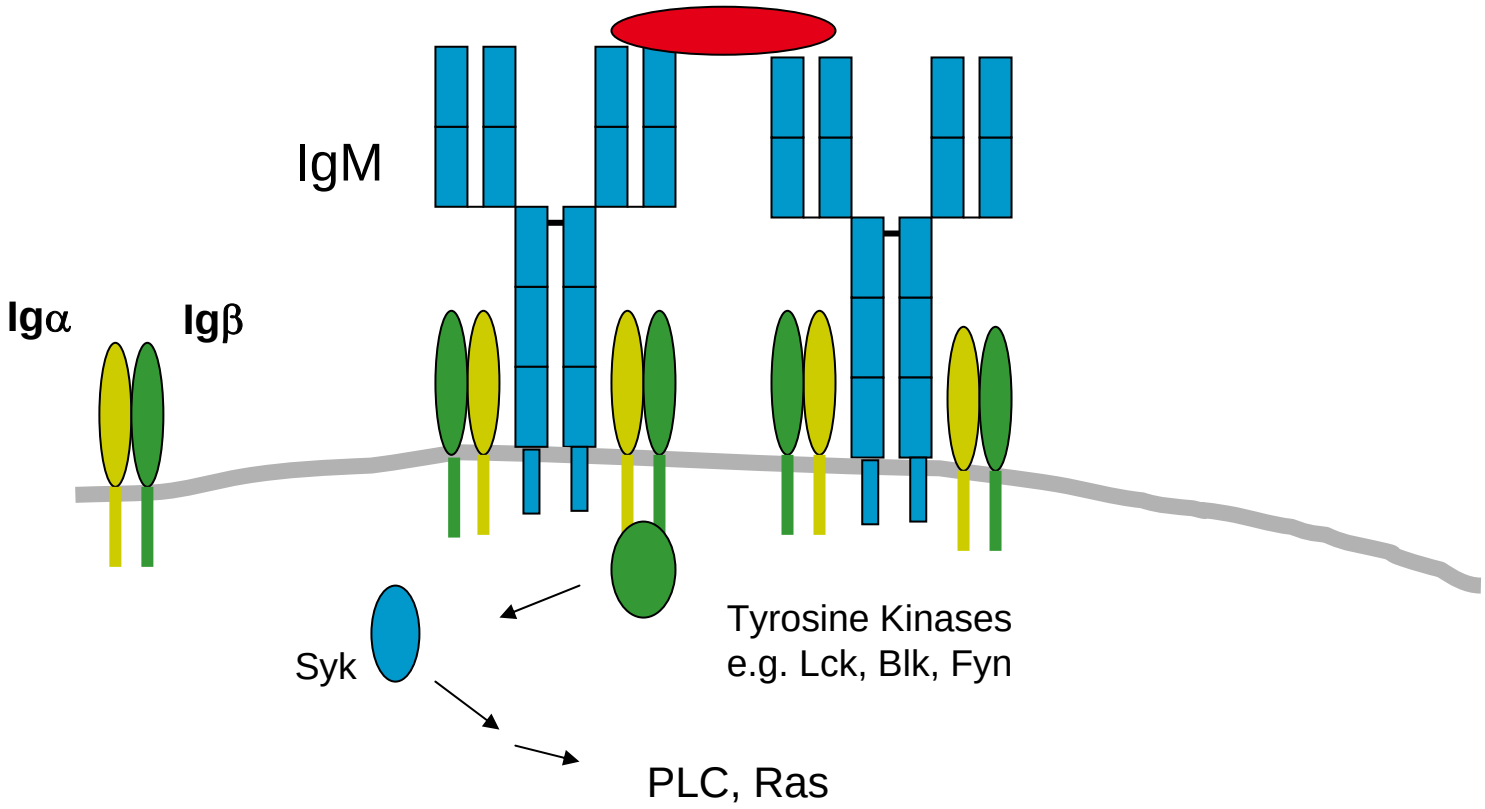
B Lymphocyte (B cell)



- B cells:
  - Develop from stem cells in the bone marrow and differentiate into antibody-producing plasma cells in the blood
  - Are capable of making a vast number of antibody specificities from a limited number of genes
  - Antibodies are both secreted to fight disease and get displayed on the B cell surface for antigen-specific signaling and activation

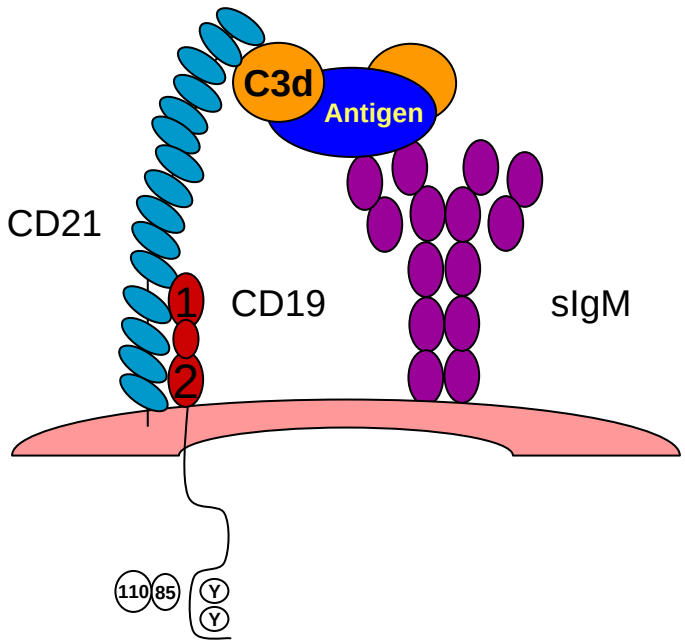
B cell receptor

Antigen can cross-link the BCR and induce a signaling cascade

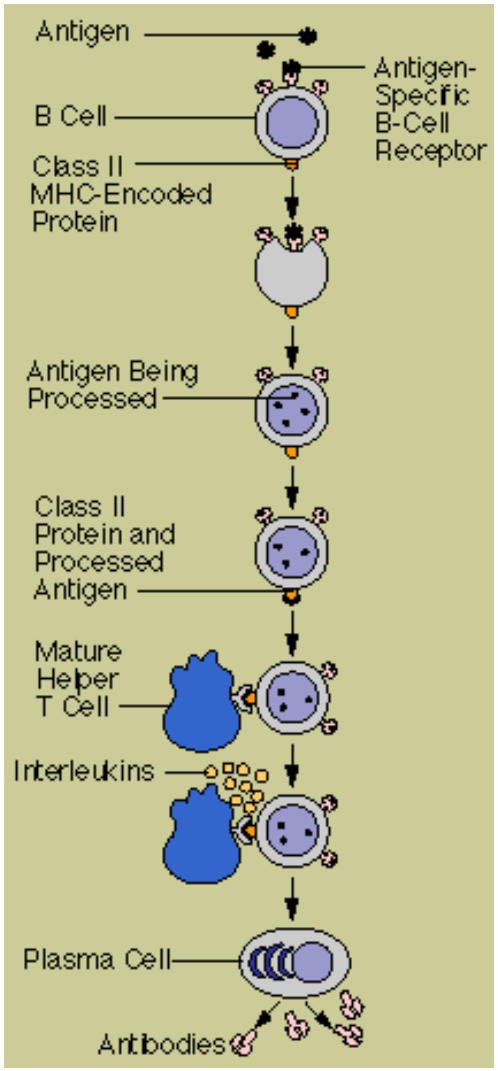


B cell co-receptor

- Lowers the threshold for cell activation in combination with antigen-specific triggering

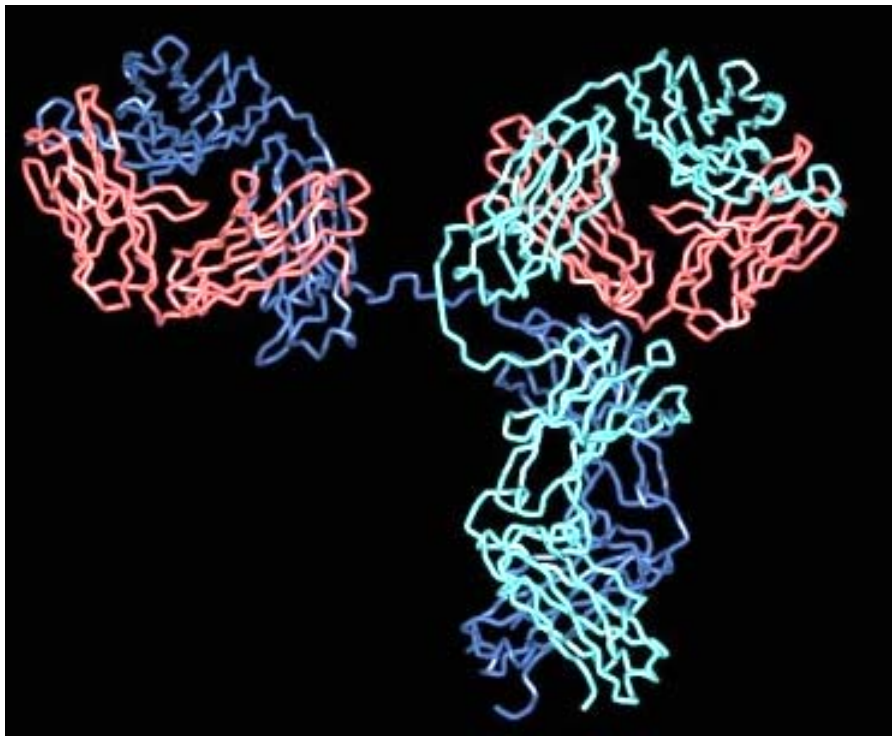


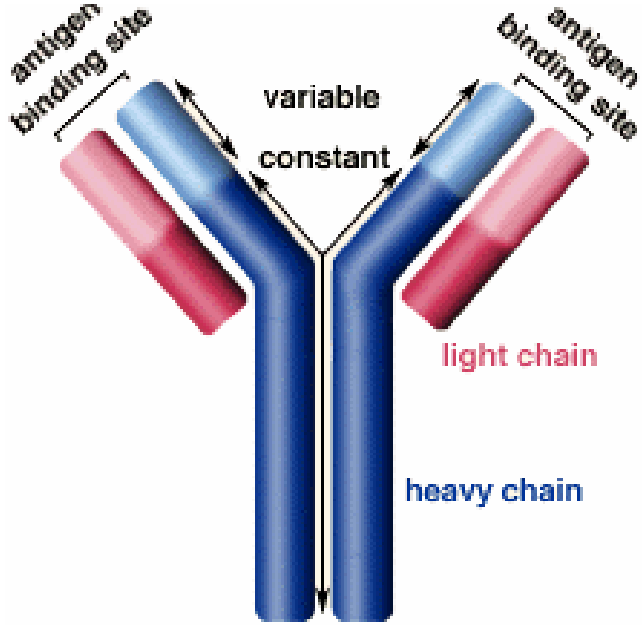
B cell activation



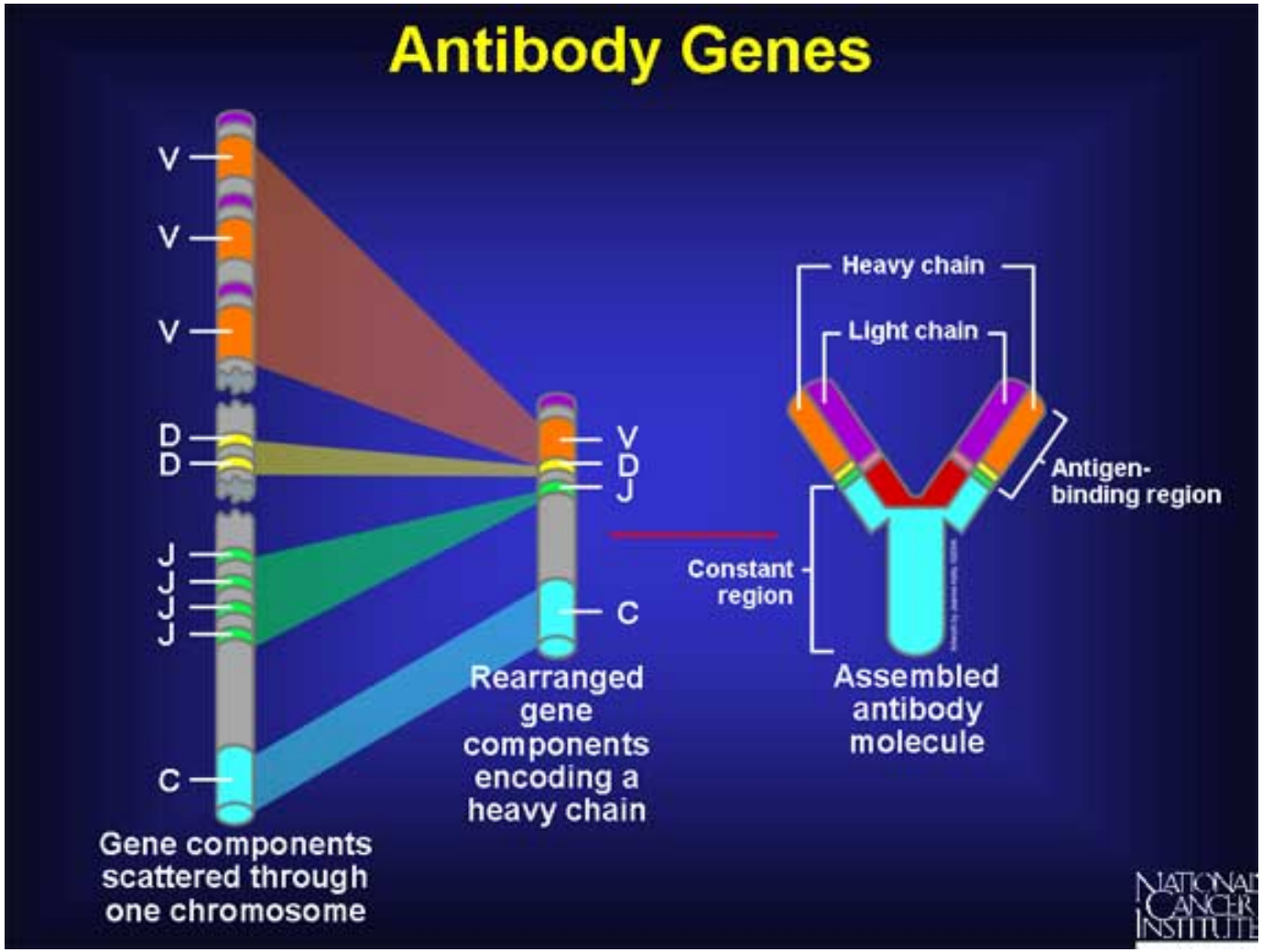
- B cells:
  - Interact with other immune cells such as helper T cells that provide essential cytokines
  - Soluble antigens bind to the B cell receptor (BCR) complex and are taken into the cell where they are degraded into peptides
  - Peptide:MHC class II complexes bind to TCR on helper T cells and stimulate cytokines essential for differentiation into antibody-producing plasma cells

Antibody Structure

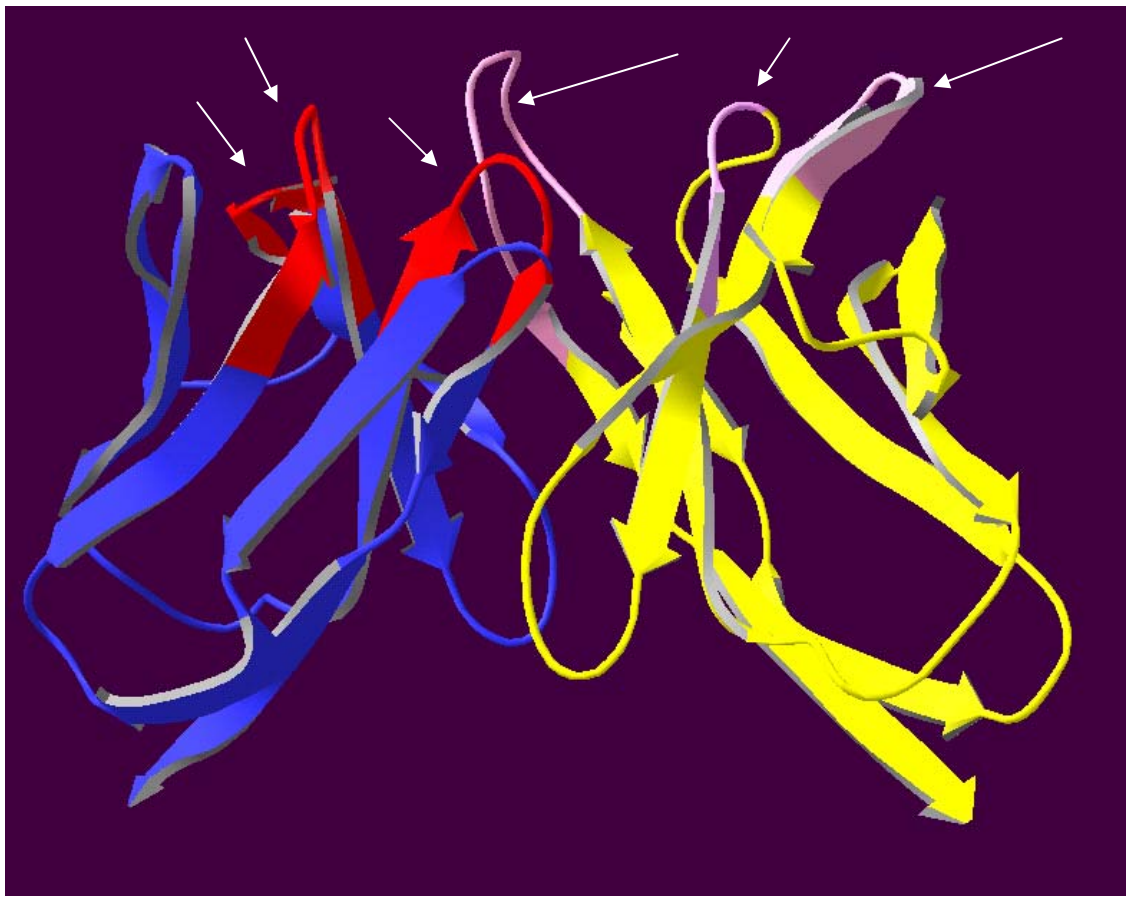




From Genes to Antibody



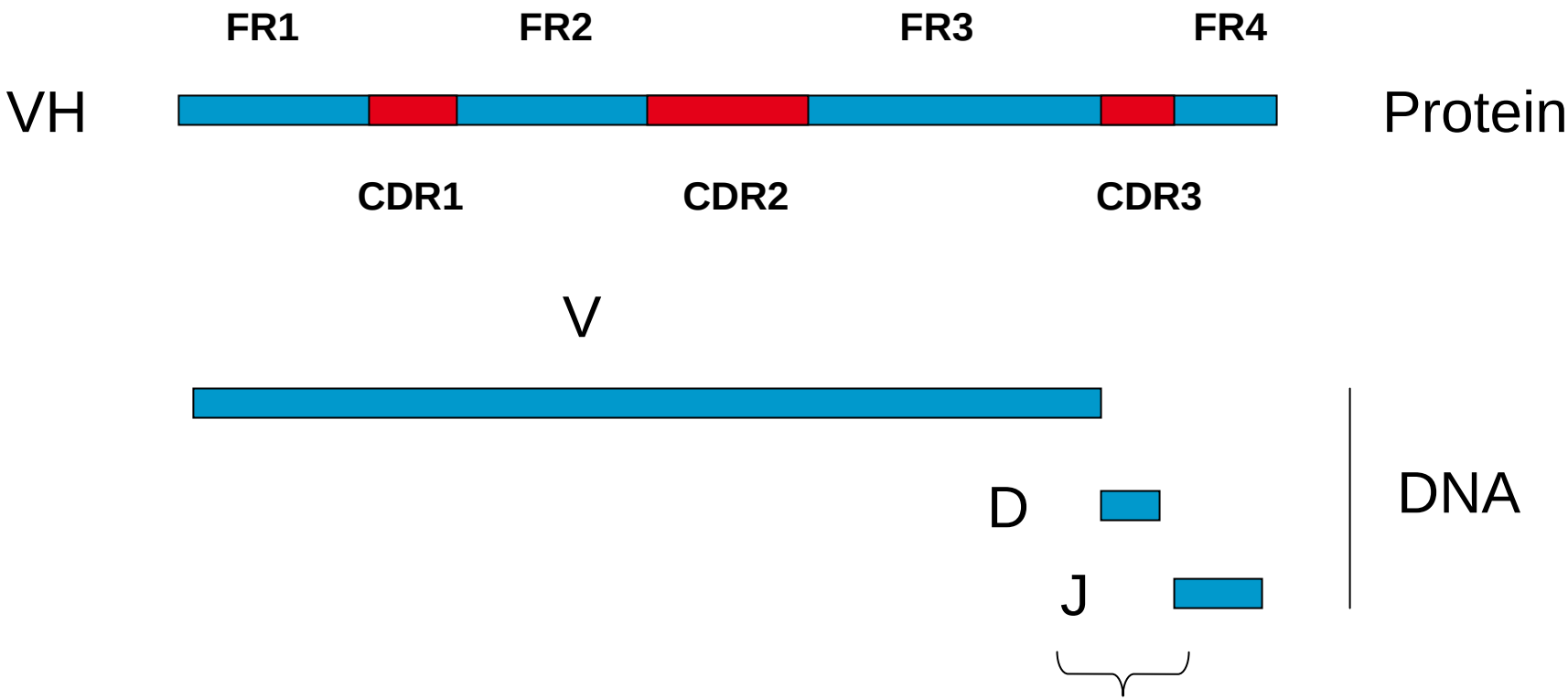
Antibody V region structure



CDRs

Antigen-binding regions

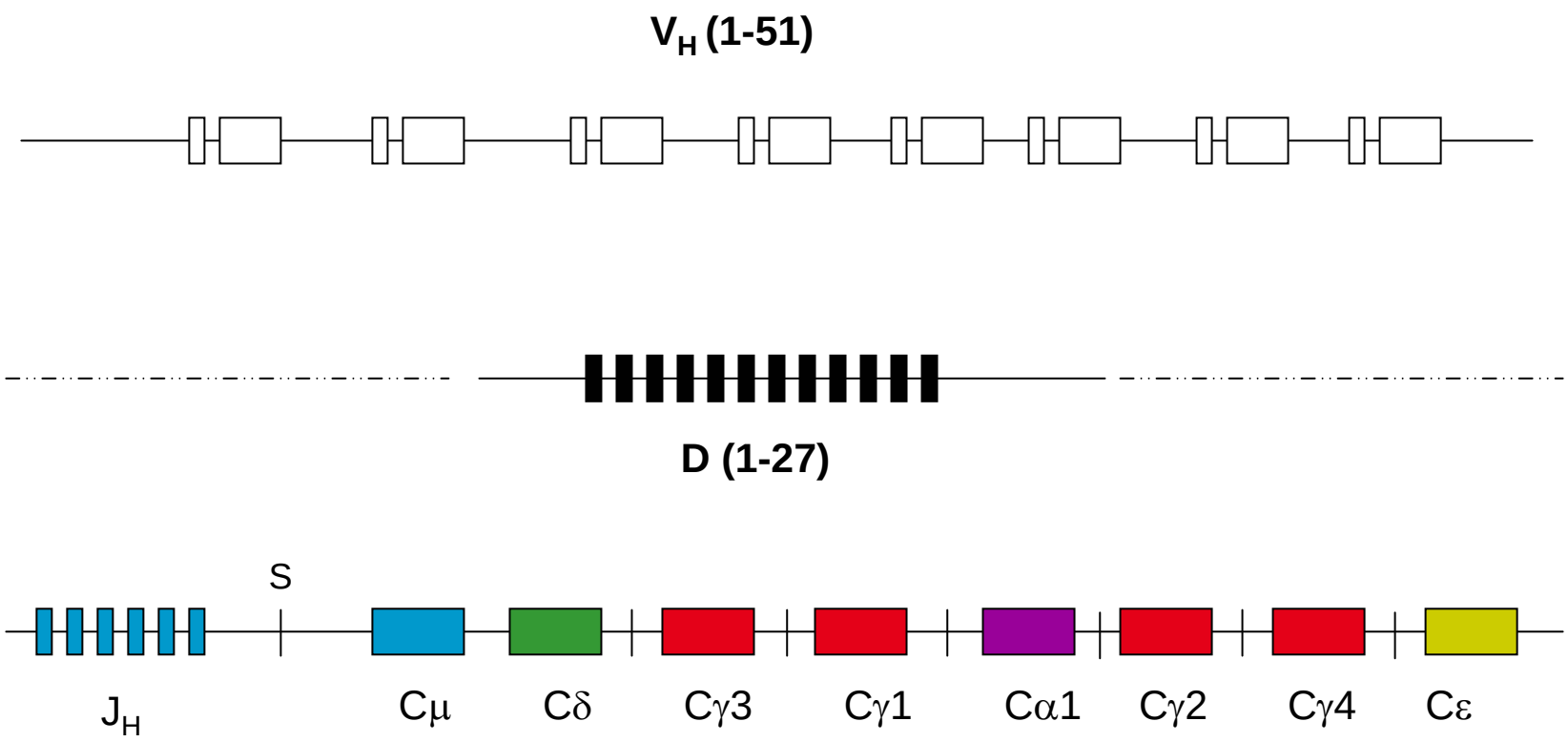
Variable regions - H chain



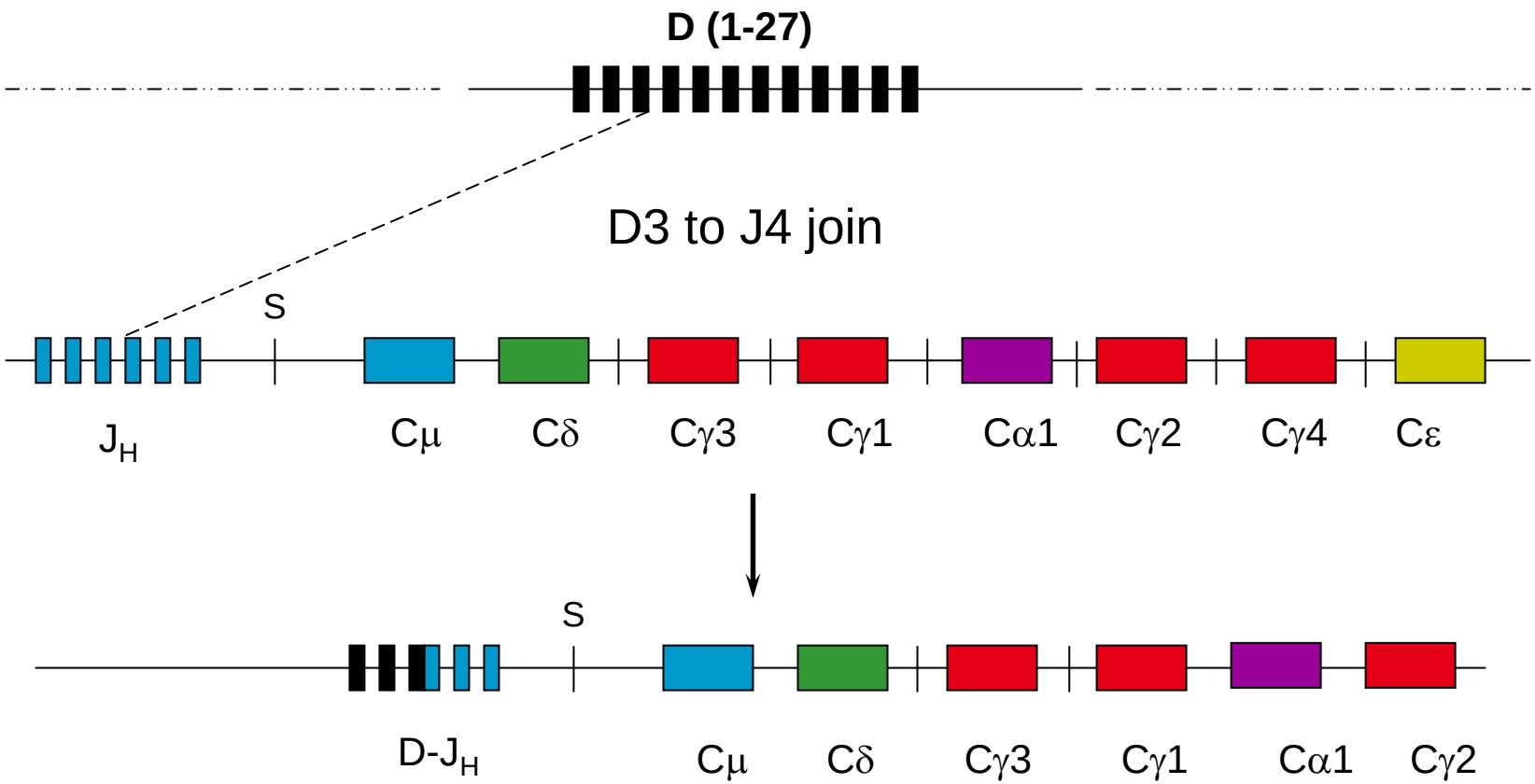
Generators of antibody diversity

- Recombination of different V + D +J regions
  - Genomic repertoire partly responsible for diversity
- Imprecision of recombination events
  - Varied cross-over events result in different D segment lengths
  - Non-template encoded N-nucleotide additions (TdT enzyme)
- Somatic hypermutation
  - Occurs when B cells respond to antigen stimulation
  - Mutations in and around CDRs

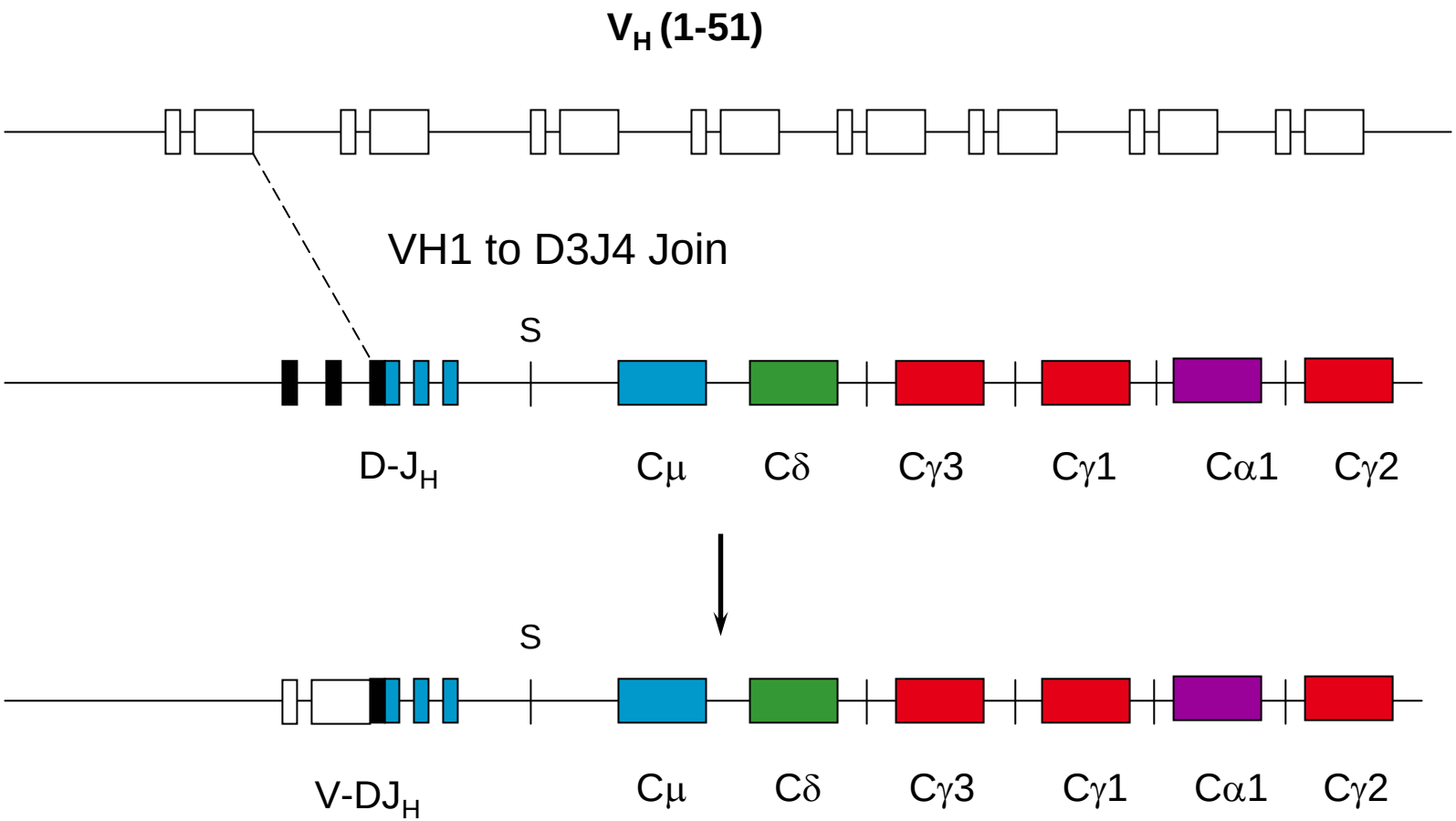
Antibody gene organization



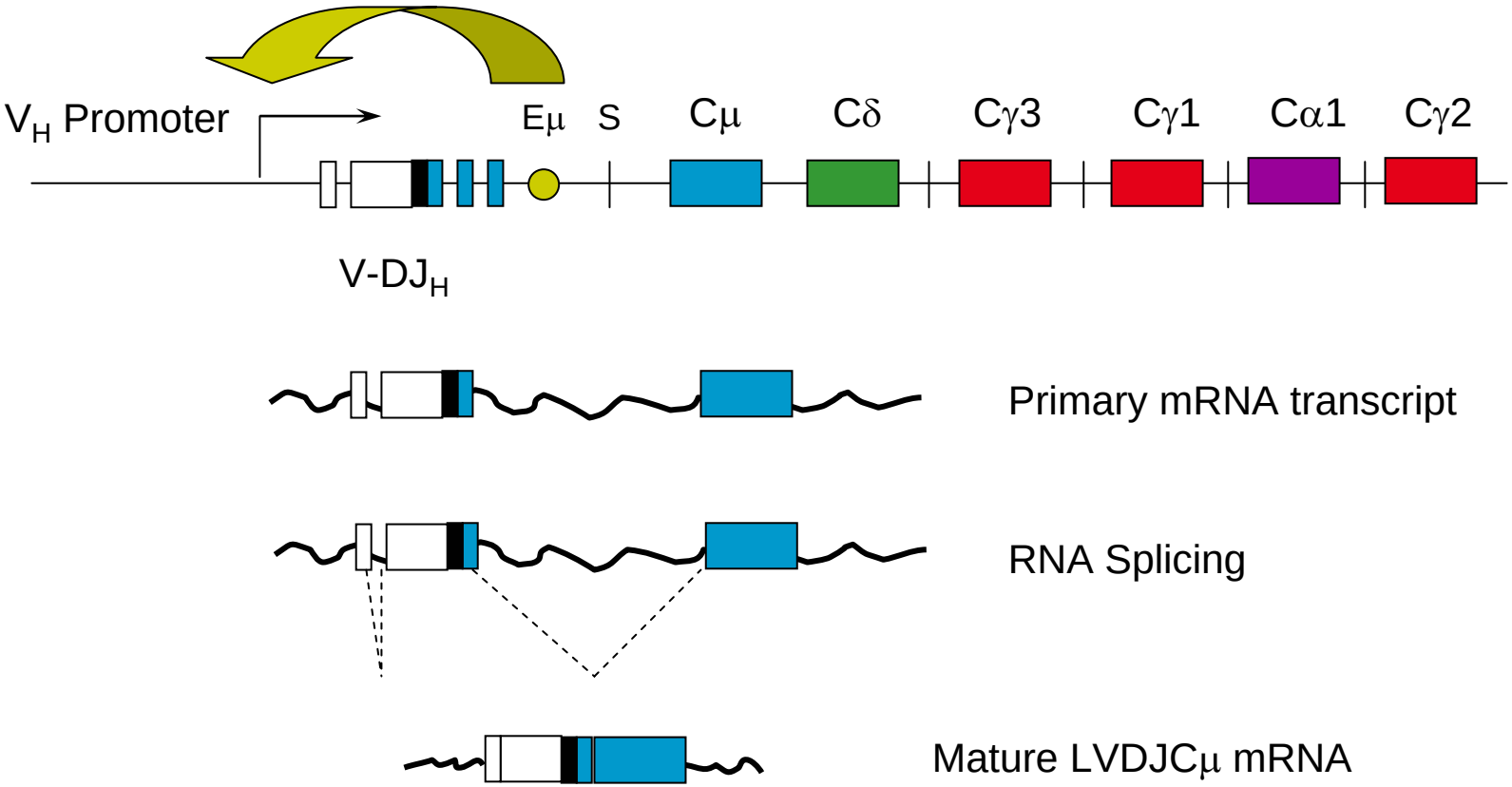
Antibody H chain gene rearrangement in **early pro-B cells**



Antibody H chain gene rearrangement in **late pro-B cells**



Productive rearrangement results in gene activation and expression



Alternative splicing creates “membrane” form of μ chain as part of BCR

Primary mRNA transcript

Alternative RNA Splicing

Addition of “membrane” exon

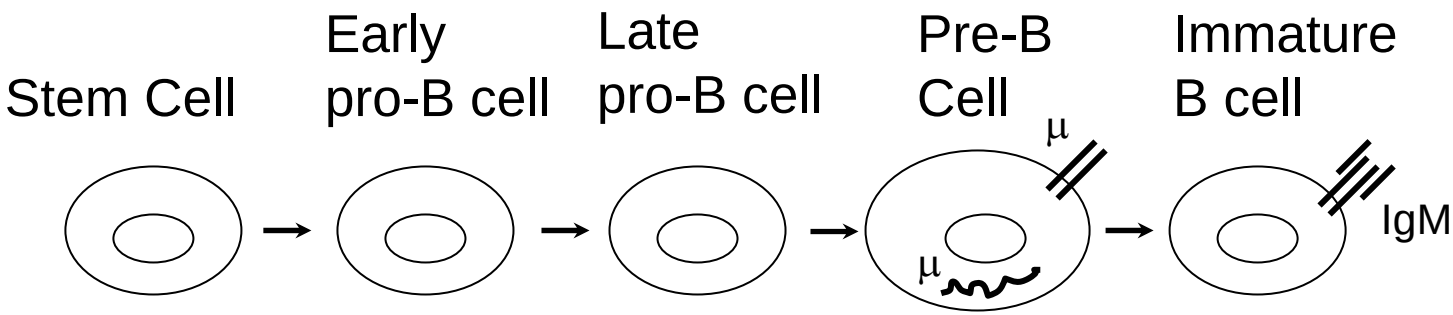
Mature LVDJCμ mRNA

Hydrophobic residues anchor into membrane

Steps in B cell development

- Antigen-independent steps
  - Occurs in bone marrow
  - H and L chain rearrangement
  - Selection of B cells with “productive” gene rearrangement
  - Self reactive clones are deleted
- Antigen-dependent steps
  - Occurs in periphery, e.g. lymph nodes
  - Expression of IgM on surface (Ag-specific BCR)
  - Additional rearrangement for isotype switch (e.g. IgG)
  - Somatic mutation drives additional diversity

Antigen-independent (early) Development

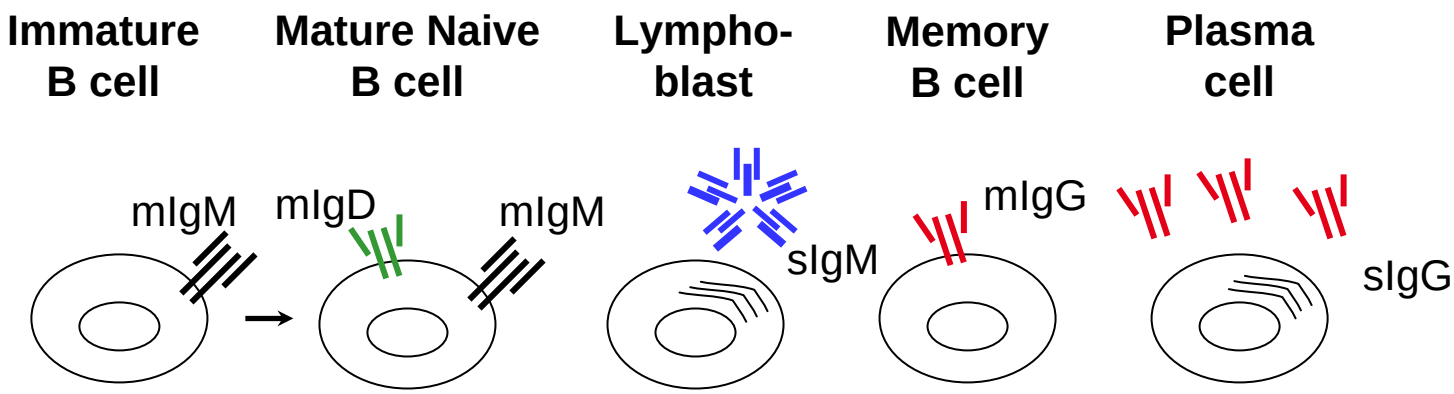


H chain	Germline	D-J Rearranged	V-DJ Rearranged	V-DJ Rearranged	V-DJ Rearranged
L chain	Germline	Germline	Germline	V-J Rearranging	V-J Rearranged
Signals	VLA4 / ICAM	Stem cell factor / Kit	IL-7 / IL7-R	IL-7 / IL7-R Other CAM	
Enzymes **Transcription factors**		Rag-1 and 2 TnT	Rag-1 and 2 TnT	Rag-1 and 2 NFκB	NFκB

Antigen-independent development – additional points

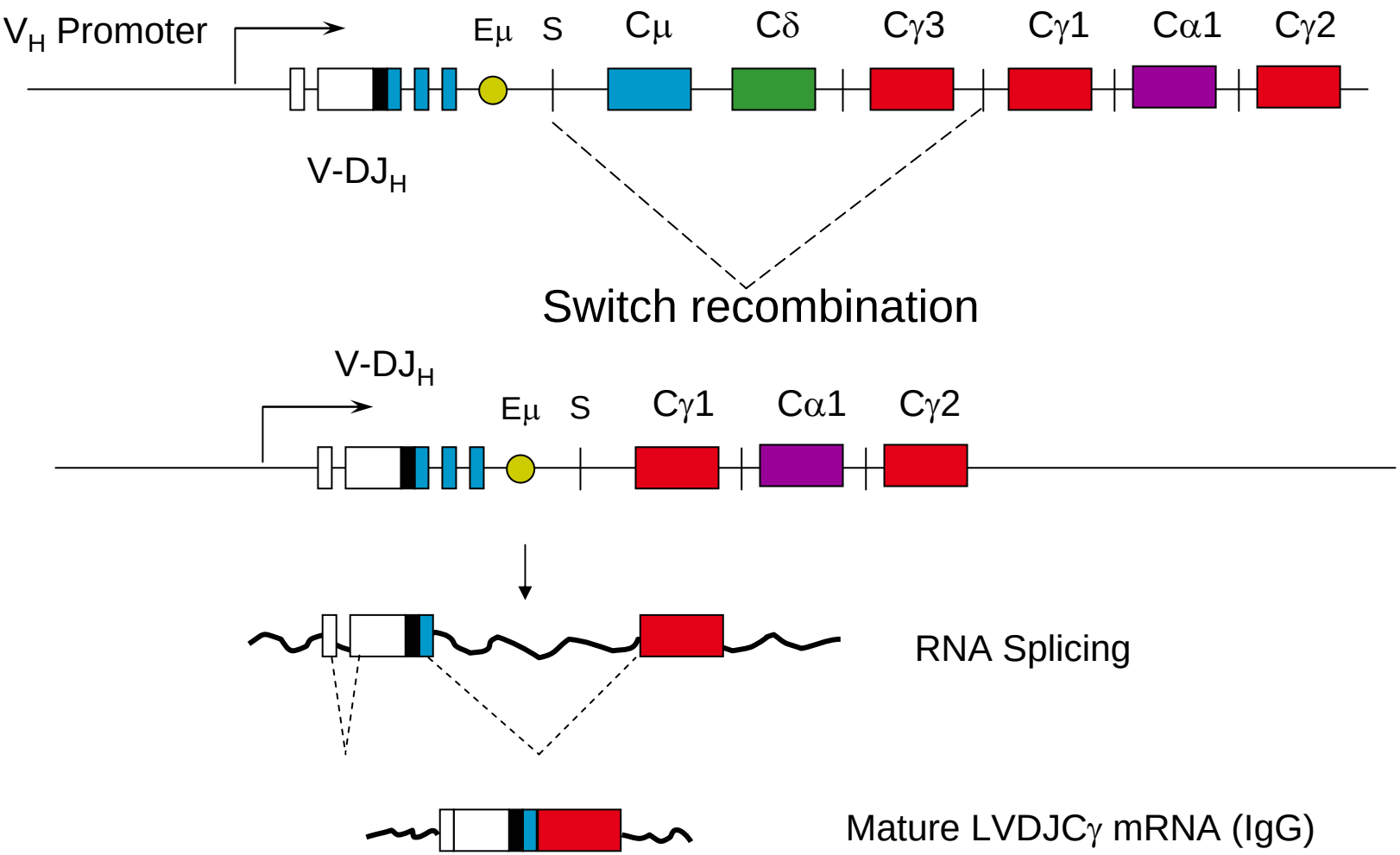
- Productive rearrangement of μ chain
  - blocks further rearrangement of H chain gene
  - Induces rearrangement of L chain genes until a successful rearrangement occurs – then further blocked
  - “Allelic Exclusion”
- Pre-B cells with expressed μ chain expand
  - Multiple chances to use a given H chain with multiple L chains

Antigen-dependent (late-stage) Development



H chain	μ chain in membrane form	μ and δ chains in membrane form via alternative splicing	Alternative splicing of μ to secreted form	Somatic hyper mutation Isotype switch to Cγ, Cα, Cε	Isotype switching and alternative splicing to secreted Ab
L chain	V-J Rearranged	V-J Rearranged	V-J Rearranged	V-J Rearranged Somatic hyper mutation	

Isotype switching occurs by recombination and gene deletion



Consequences of isotype switching

- Change in binding valency
  - Pentameric IgM ➔ Dimeric Ig
  - Compensated by affinity maturation
- Change in antibody properties
  - Circulating half-life IgG > IgM > IgE
  - Effector functions - ADCC, complement fixation
  - Biodistribution
    - IgG blood
    - IgA mucosal surfaces
    - IgE mast cells

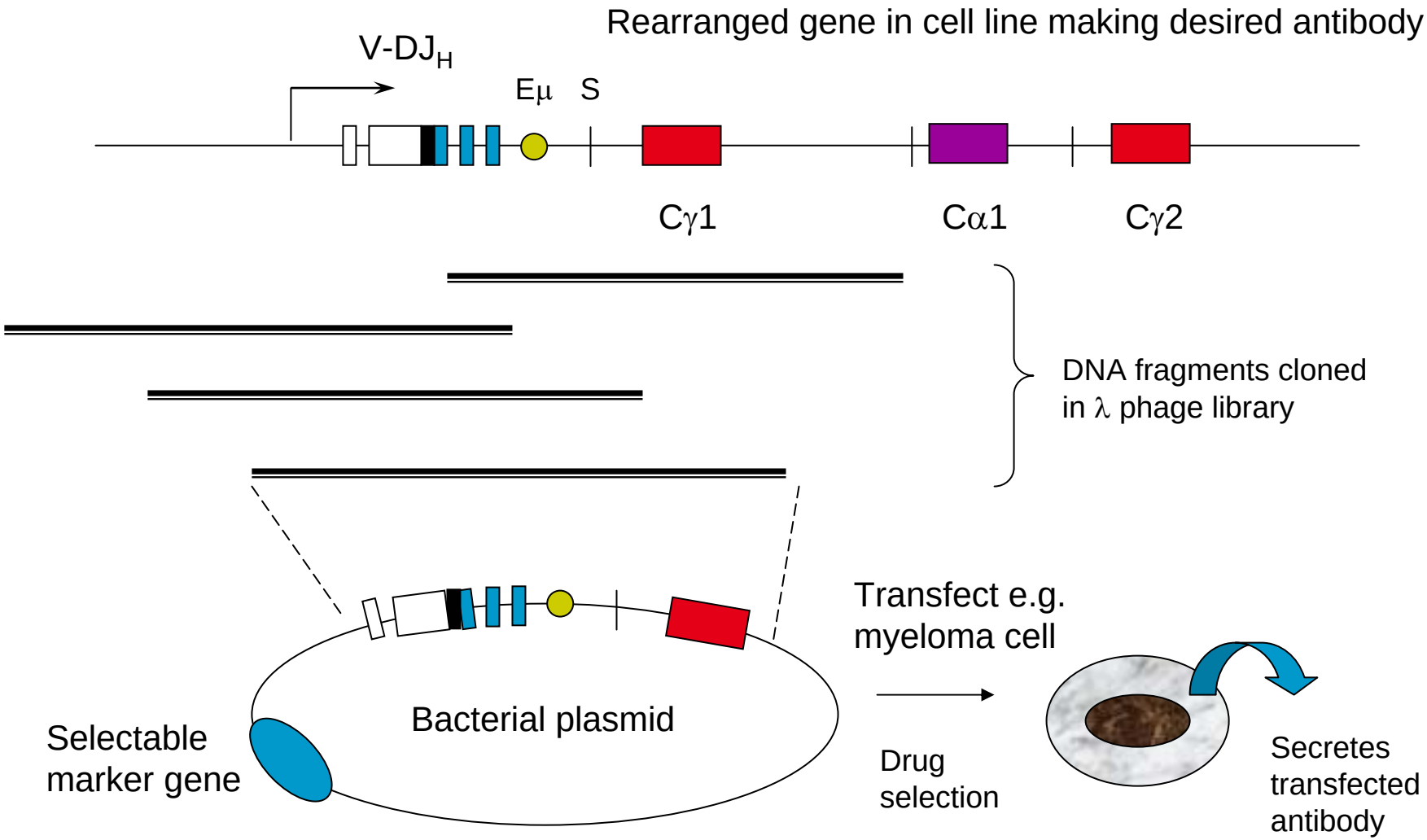
Summary

- Antibody diversity created in concert with B cell development and activation by antigen
- Involves diversity of binding
  - Affinity of Ag:Ab interaction
  - Specificity
- Also diversity of function
- Nature has been performing Antibody Engineering
- Knowledge gained inspired genetic engineering of Abs

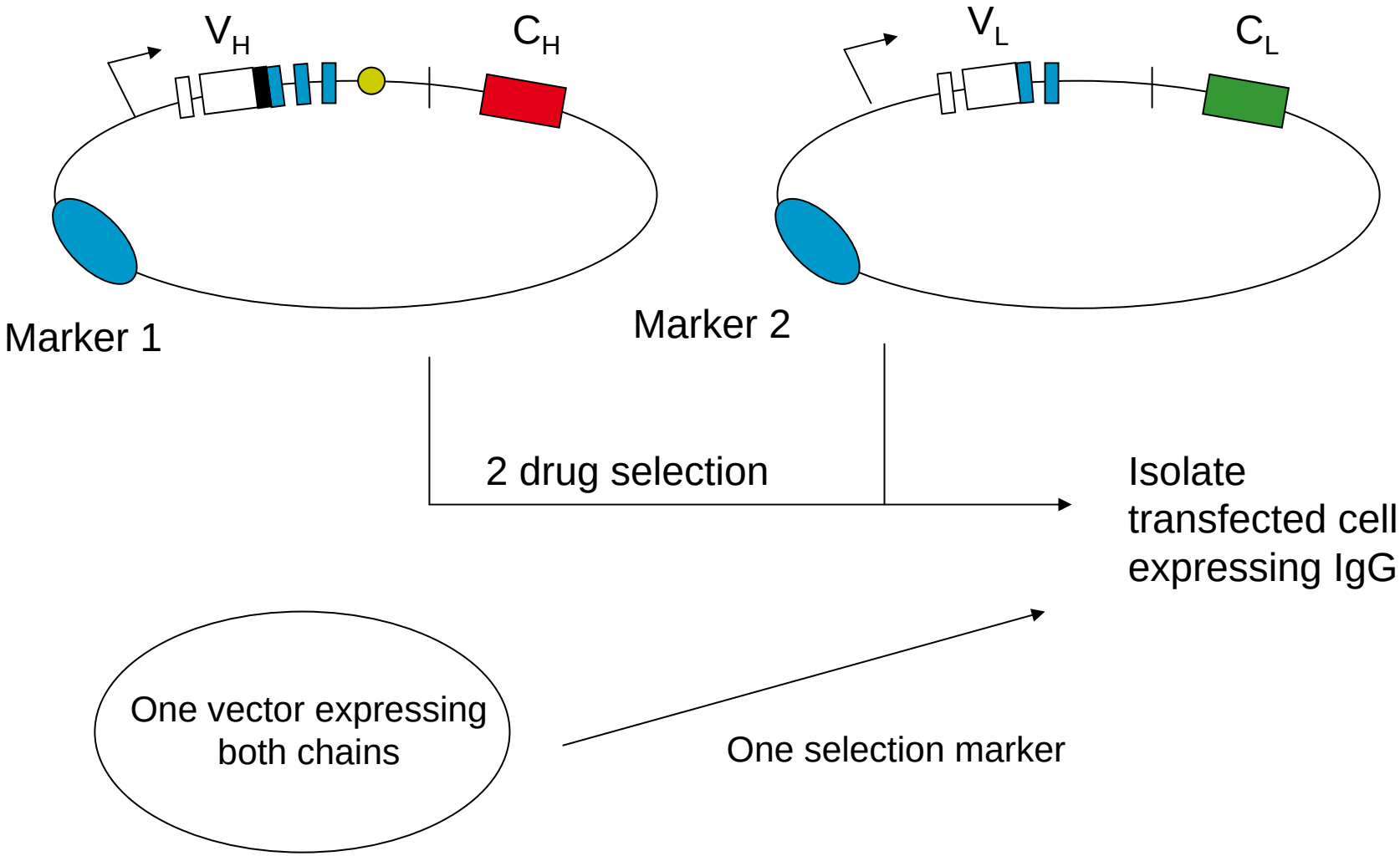
Antibody Engineering

- First step - development of monoclonal antibodies
  - Fusion of antibody-producing B cell with myeloma
  - Results in immortalized monospecific Ab-producing cell line
- Second step - ability to clone and re-express Abs
  - Initially done with cloned, rearranged genes from hybridomas
  - Parallel work with isolated Fab fragments in bacteria
- Third step - re-engineering for desired properties
  - Reducing immunogenicity of mouse antibodies
  - Tailoring size and half-life for specific need
  - Adding or removing functions
- Engineered diversity – phage display approach

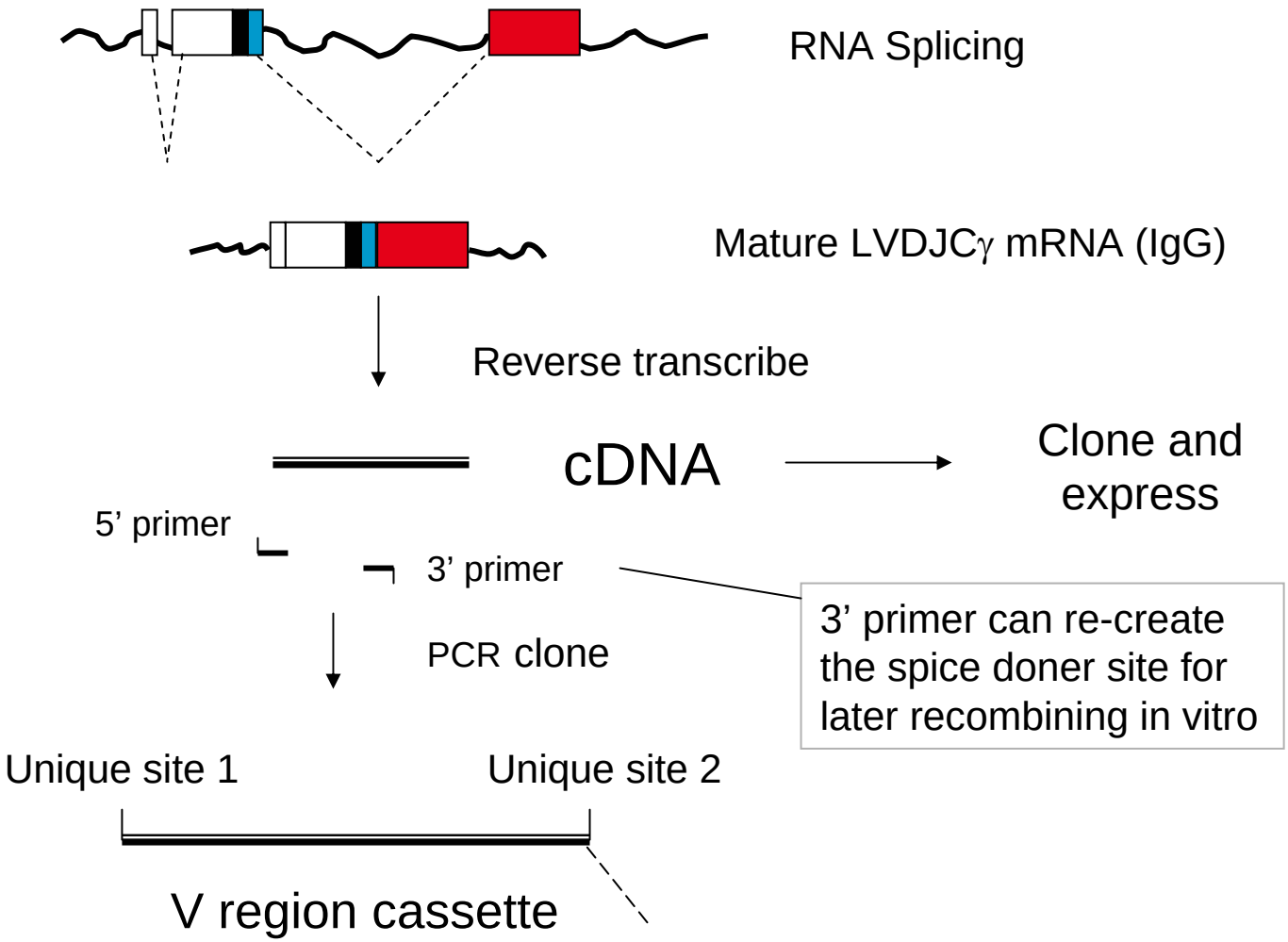
Cloning and re-expressing antibodies



Cloning and re-expressing antibodies

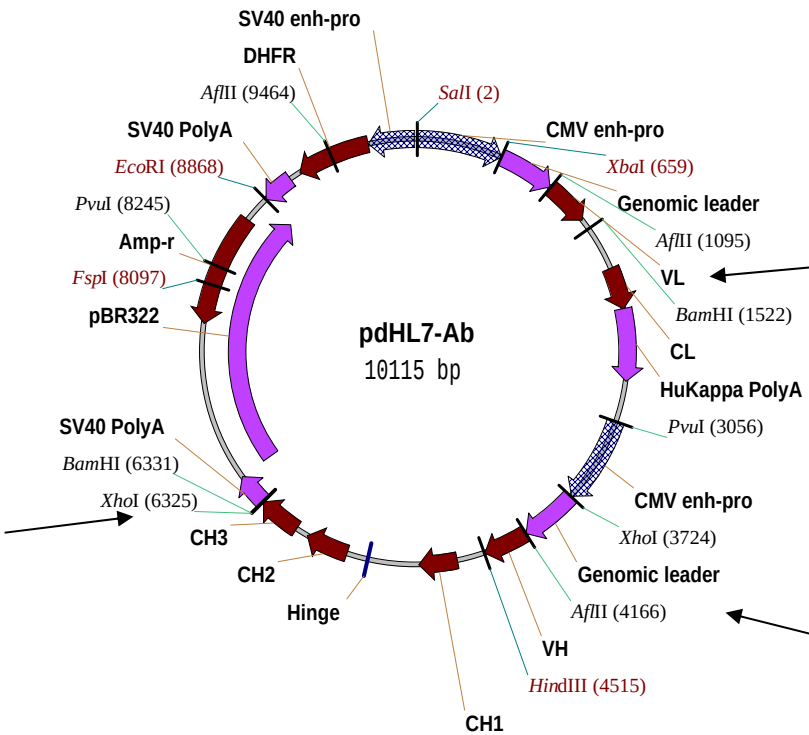


More efficient cloning



Single vector for high level Ab expression in lymphoid or non-lymphoid cells

- V_H and V_L cassettes are inserted into pre-designed sites of modular expression vector
- Modular design allows for easy isotype or fusion protein switching
- Promoter/enhancers optimized for multiple cell types
- High producers obtained by gene amplification or forced selection of optimal integration

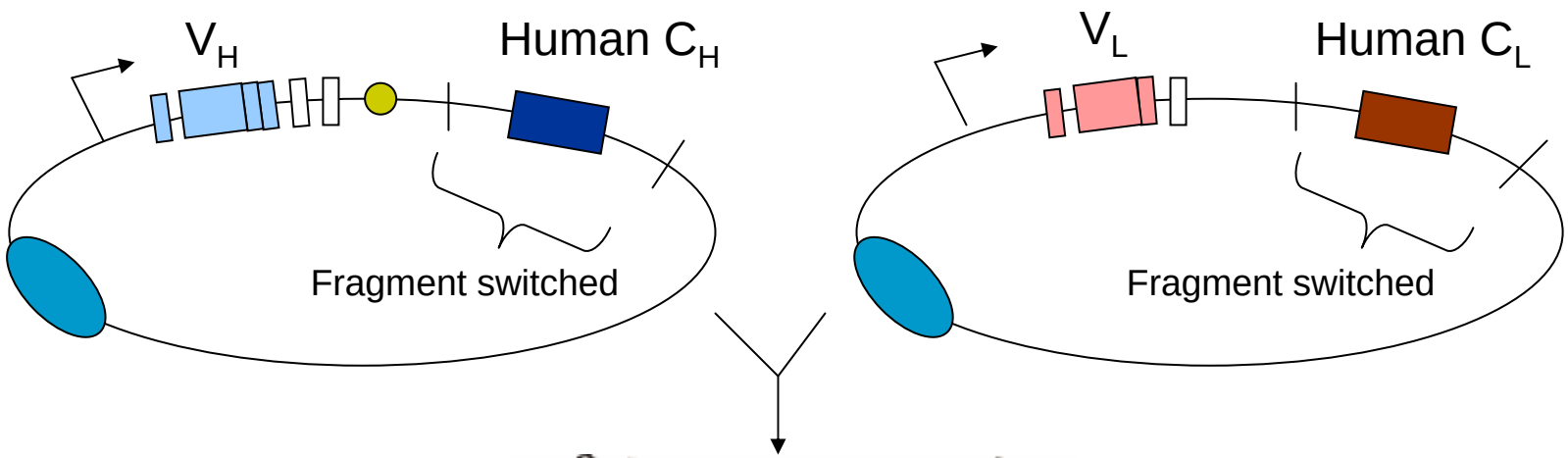


V_L insertion

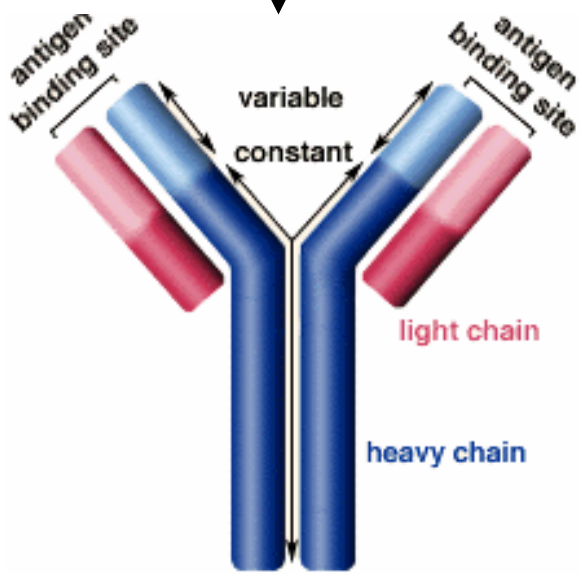
Fusion protein insertion site

V_H insertion

Chimeric Mouse-human antibodies



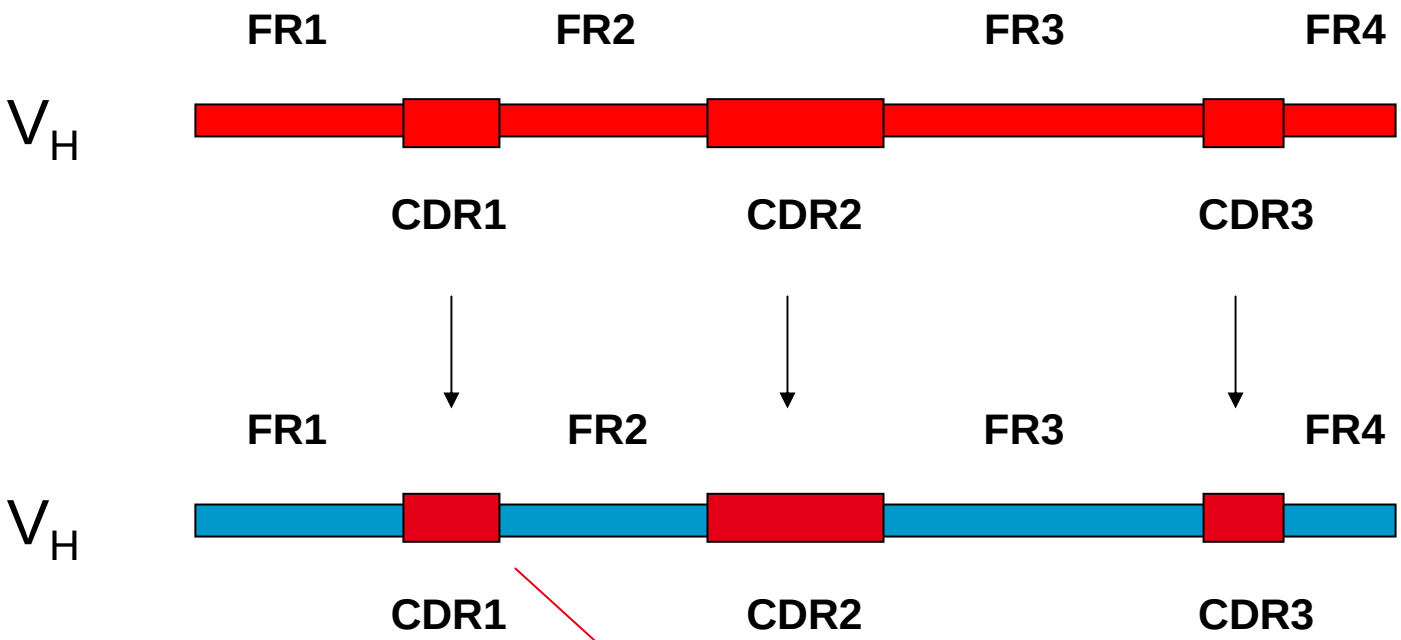
e.g. Erbitux

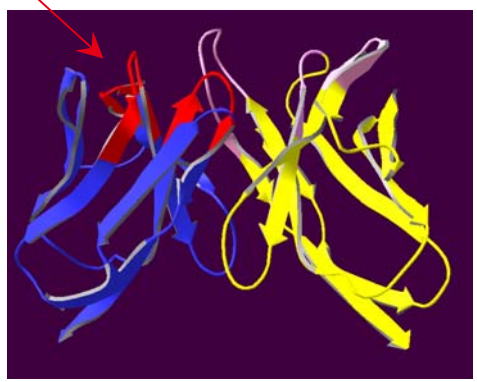


Mouse-derived

Human-derived

V Region “humanization”



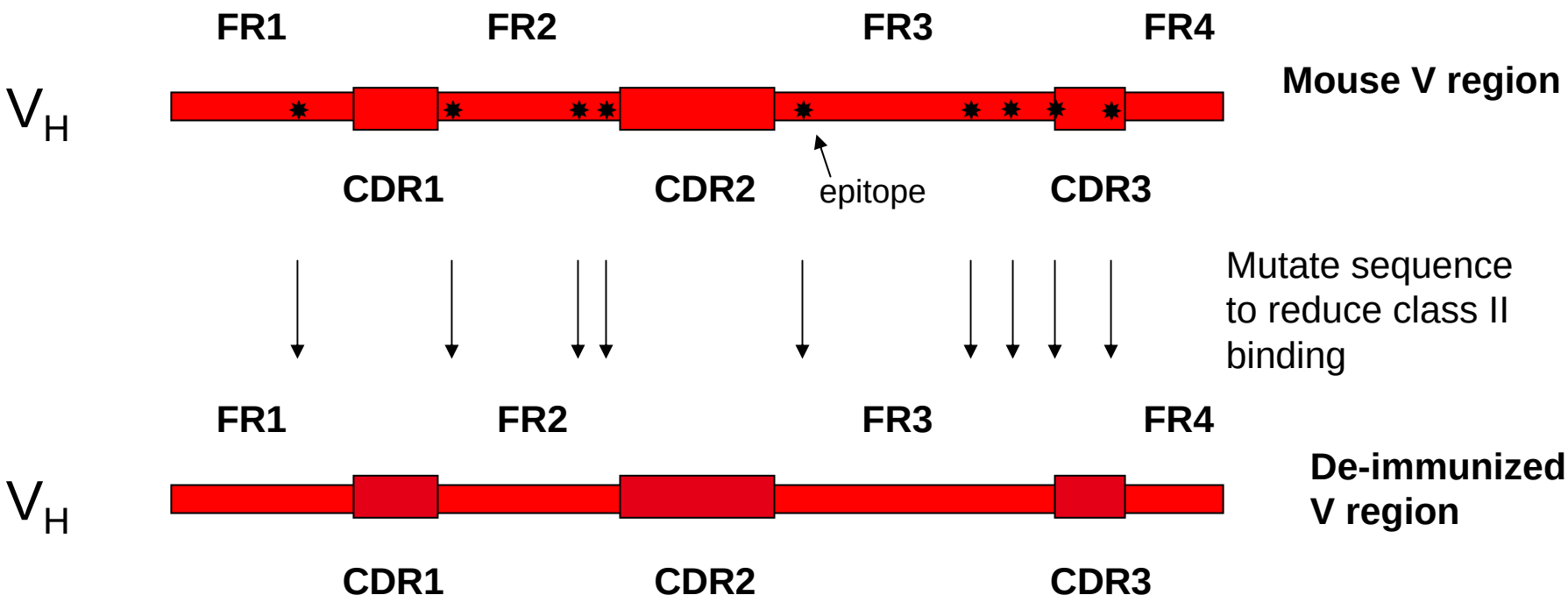


This approach has worked but often leads to mis-folding of CDRs requiring additional FR back-mutations

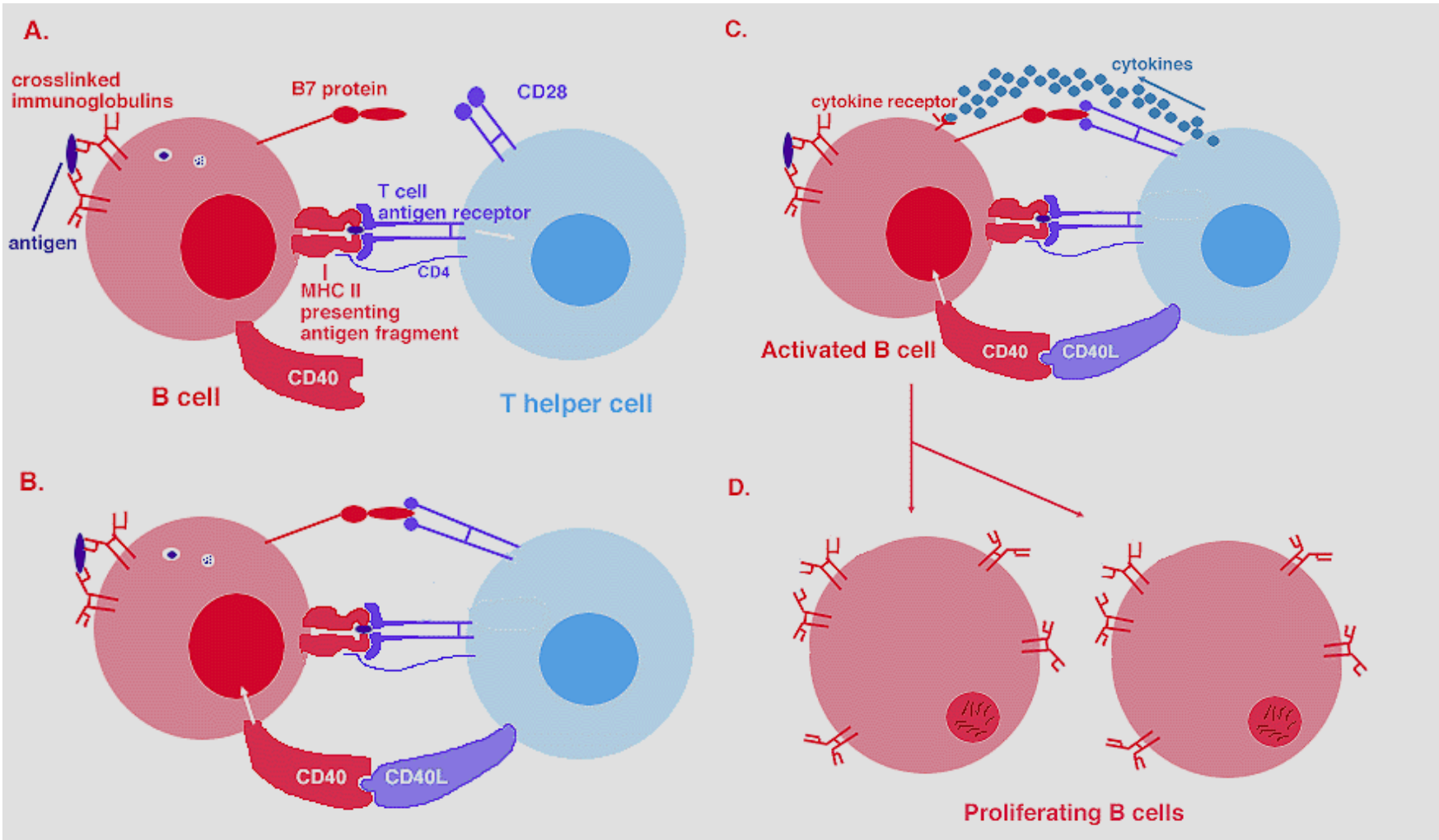
Is “humanization” what we want

- No – we want the protein to be non-immunogenic
- Humanized and even human V regions can contain T cell epitopes that differ from the germline (via somatic mutation or recombination events)
- What is “self” for a V region sequence donor can be “non-self” for the general population

V Region “de-immunization”

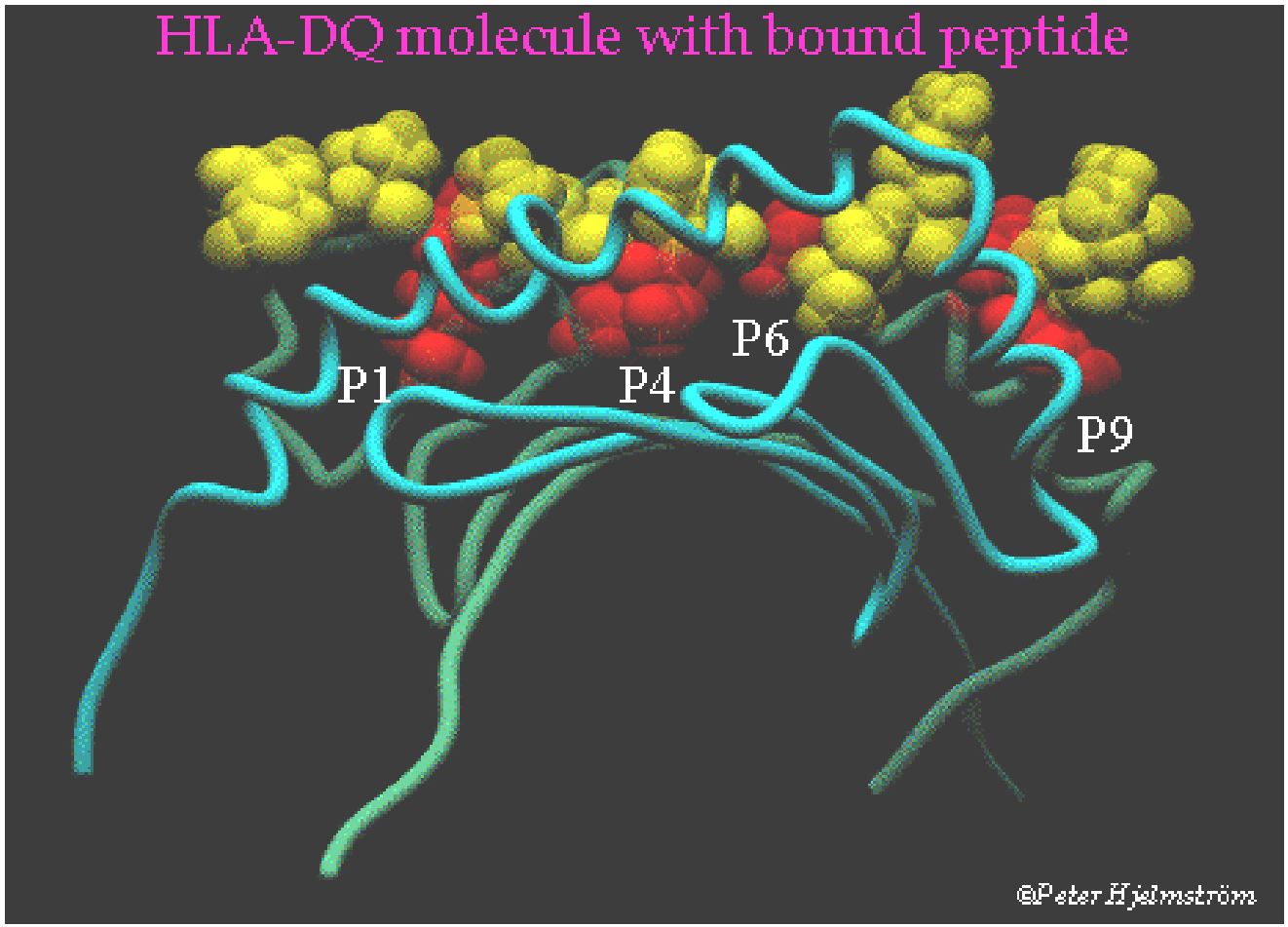


Antibody production requires MHC Class II antigen presentation



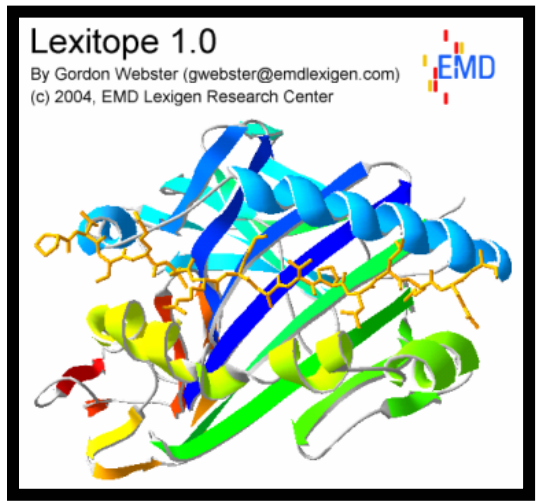
MHC Class II Peptide Binding

What we‘re actually targeting in deimmunization

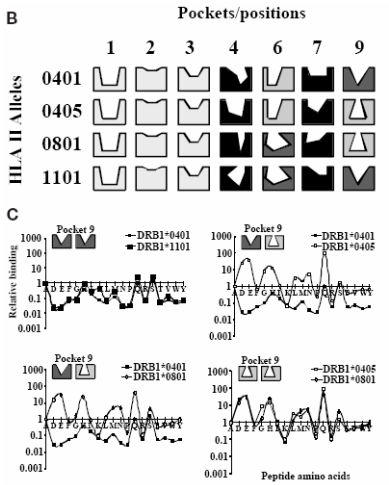


Protein Deimmunization Based Upon *in silico* Prediction Of T-cell Epitopes

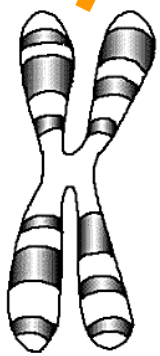
Immunogenic Protein



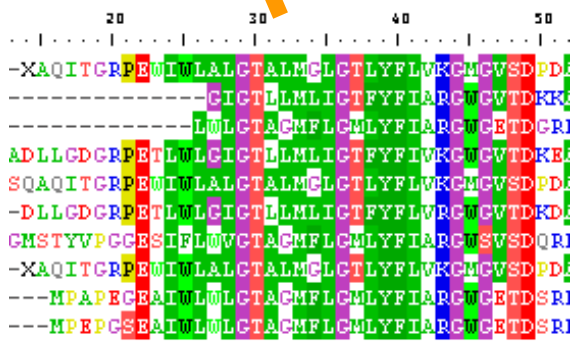
Deimmunized Protein



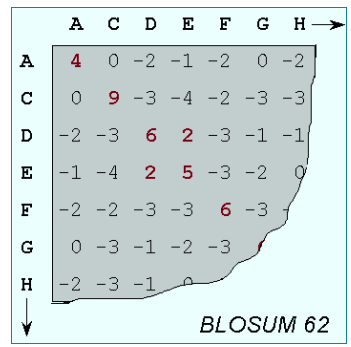
HLA Binding Matrices



Germlines



BLAST Alignments



Substitution Matrices

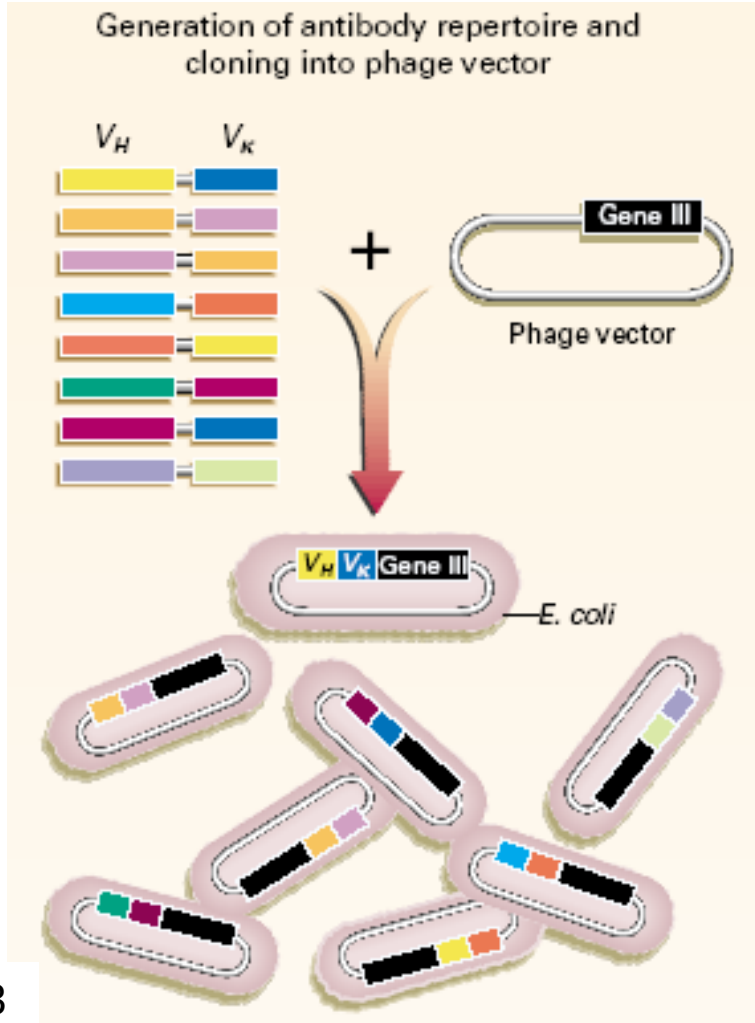
Artificial Ab libraries

- Phage-display methods (pros)
  - Allow for rapid screening of huge numbers (high diversity)
  - Are not biased against “self” binders due to human-mouse homology
  - Can be used for affinity maturation
  - Can be used to isolate human antibody V region binders
- Phage-display methods (cons)
  - Lack of in vivo editing for cross-reactivity
  - May still contain immunogenic epitopes
  - Costly to commercialize

Phage library construction

Different sources of V gene input:

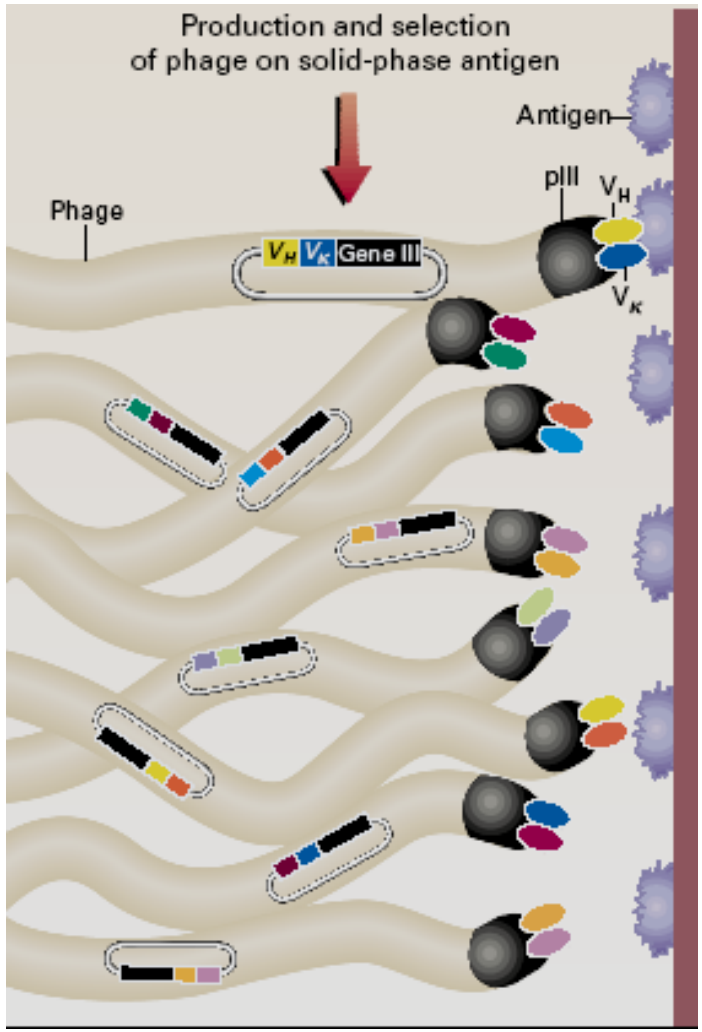
- cDNA from immune or naïve individual
- germline V regions



Marks, NEJM 335:730-733

Phage antibody selection

Phage library is expanded and generated from infected E. coli.



Individual phage can be isolated from plaques and expanded for further testing.

↓

DNA encoding desired V region can be derived by PCR from the “monoclonal” phage particle.

↓

Whole antibody can be re-constructed from phage V regions

Marks, NEJM 335:730-733

Therapeutic Antibody Approaches

- Whole Antibodies (chimeric, human, de-immunized, etc.)
  - Antagonists
    - Anti-EGFR, CTLA4
  - Agonists
    - Anti-CD40
  - Anti-virals
    - Neutralizing RSV
  - Depleting immune cells
    - Anti-CD20, CD19, CD22, CD3, CD4

Therapeutic Antibody Approaches

- Antibodies as carriers
  - Radio-immune therapy (Zevalin, Bexxar)
  - Immunotoxins
  - Antibody-drug conjugates
  - Antibody-cytokine fusions
- Bi-specific antibodies
  - Dual specificity (same cell)
  - Cross-linking cells (e.g. anti-T cell / anti-tumor cell)