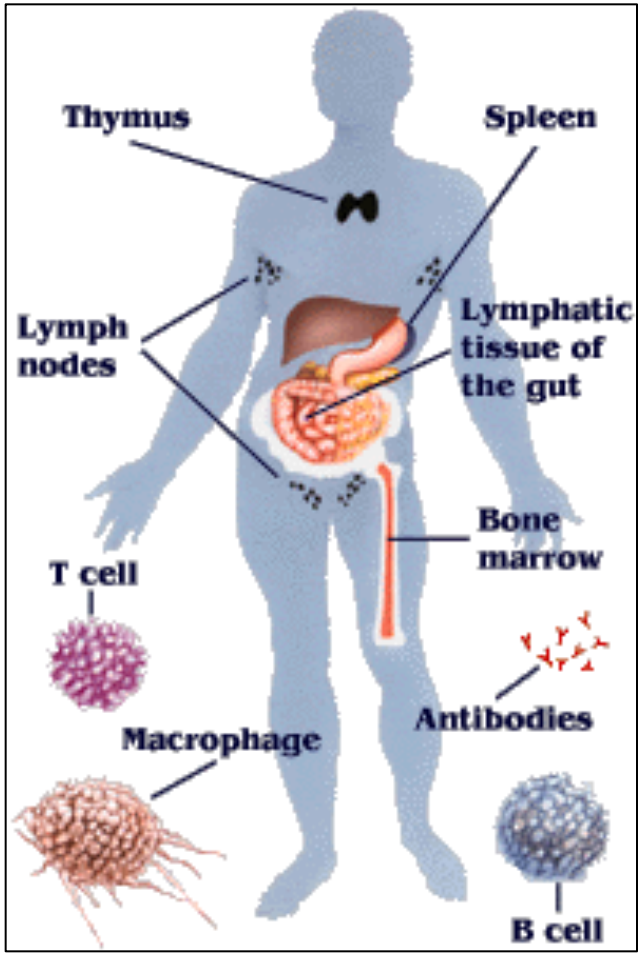
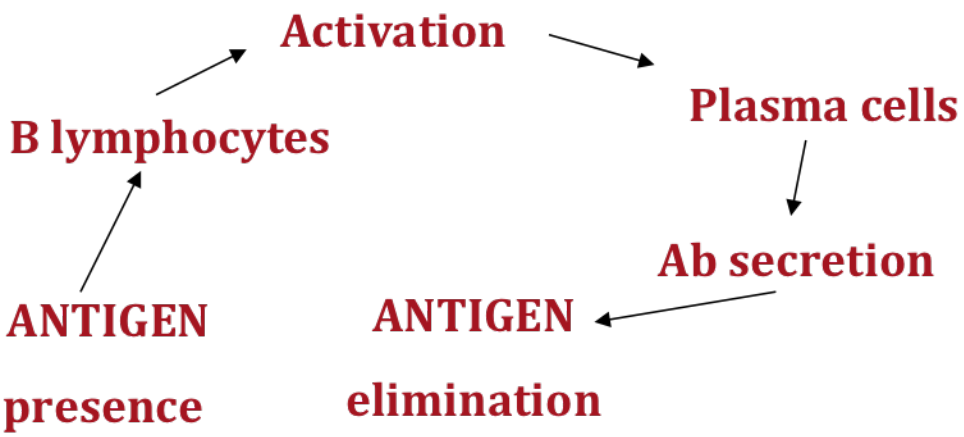
Human antibodies

General informations



- Innate Immunity (natural)
  - Humoral
  - Cell-mediated
- Acquired Immunity (specific)
  - Humoral
  - Cell-mediated

Antibodies are the most important component of humoral immune system.



Glossary

Antibody (anti-foreign body) is a glico-protein produced by a white cell (B lymphocyte and plasma cells).

Antigen (antibody generating substance) is any agent, such as a chemical or microorganism that is recognized by the antibody.
Not all antigens are immunogens (e.g hapten).

Immunogen: Any substance to which an animal responds by making antibodies. All immunogens are antigens.

Immunogenicity

Ability of a molecule to induce an immune response

Proteins, peptides, carbohydrates, nucleic acids, lipids

Must be larger than 3000-5000 daltons - if not…..

Antigen (hapten) → Carrier protein e.g. BSA Thyroglobulin

Carbodiimide

Glutaraldehyde

MBS–Heterobifunctional reagents

Glossary

Antibody (anti-foreign body) is a protein produced by a white cell (B lymphocyte).

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Epitope : the part of a target to which an antibody binds, also known as an antigenic determinant

Glossary

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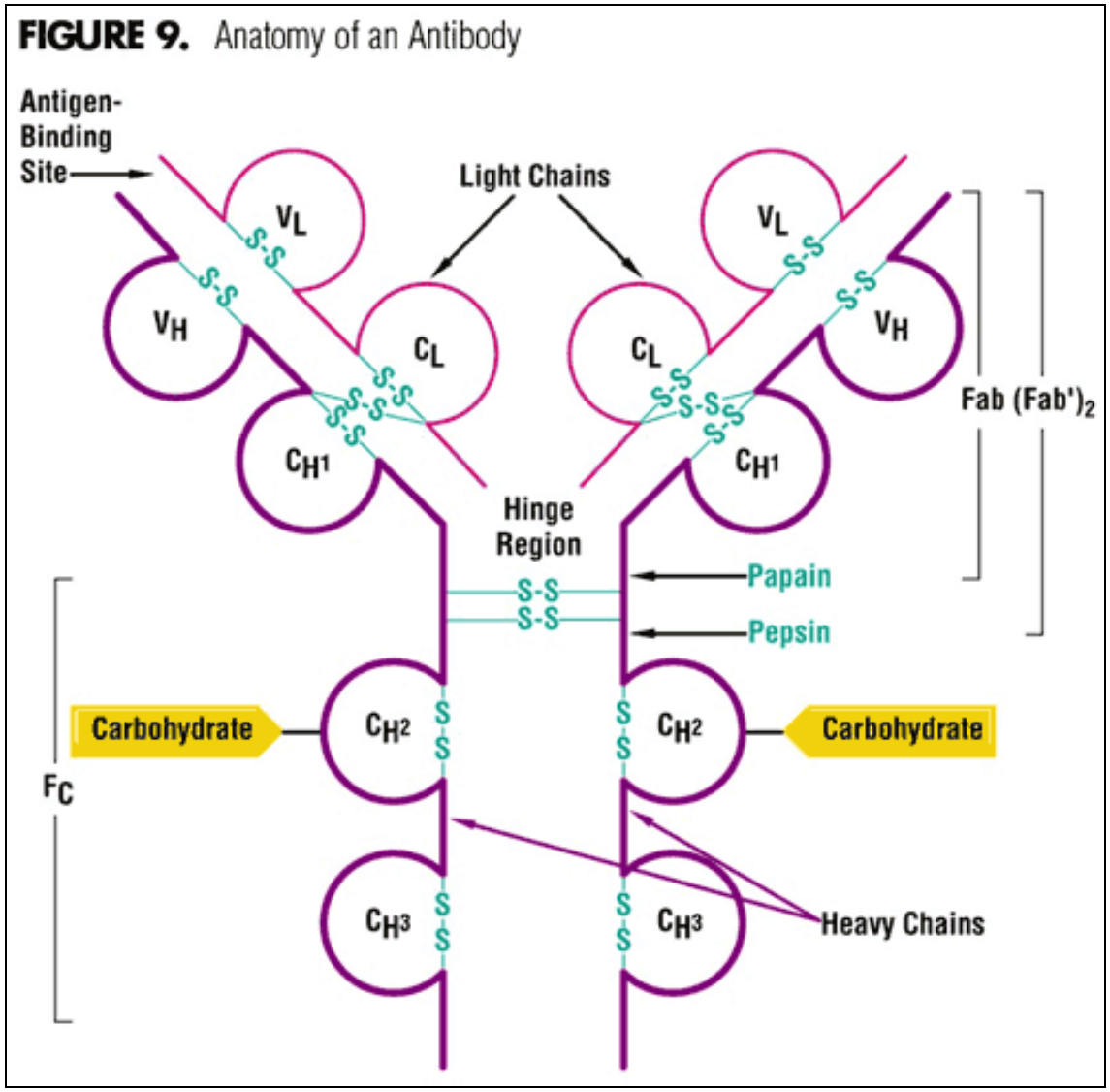
Epitope : the part of a target to which an antibody binds, also known as an antigenic determinant

Antigen binding site - relatively small region of an antibody that binds to the antigen.

ANATOMY OF AN ANTIBODY



2 HEAVY CHAINS

1 VARIABLE DOMAIN (VH)

3 CONSTANT DOMAINS (CH1-2-3)

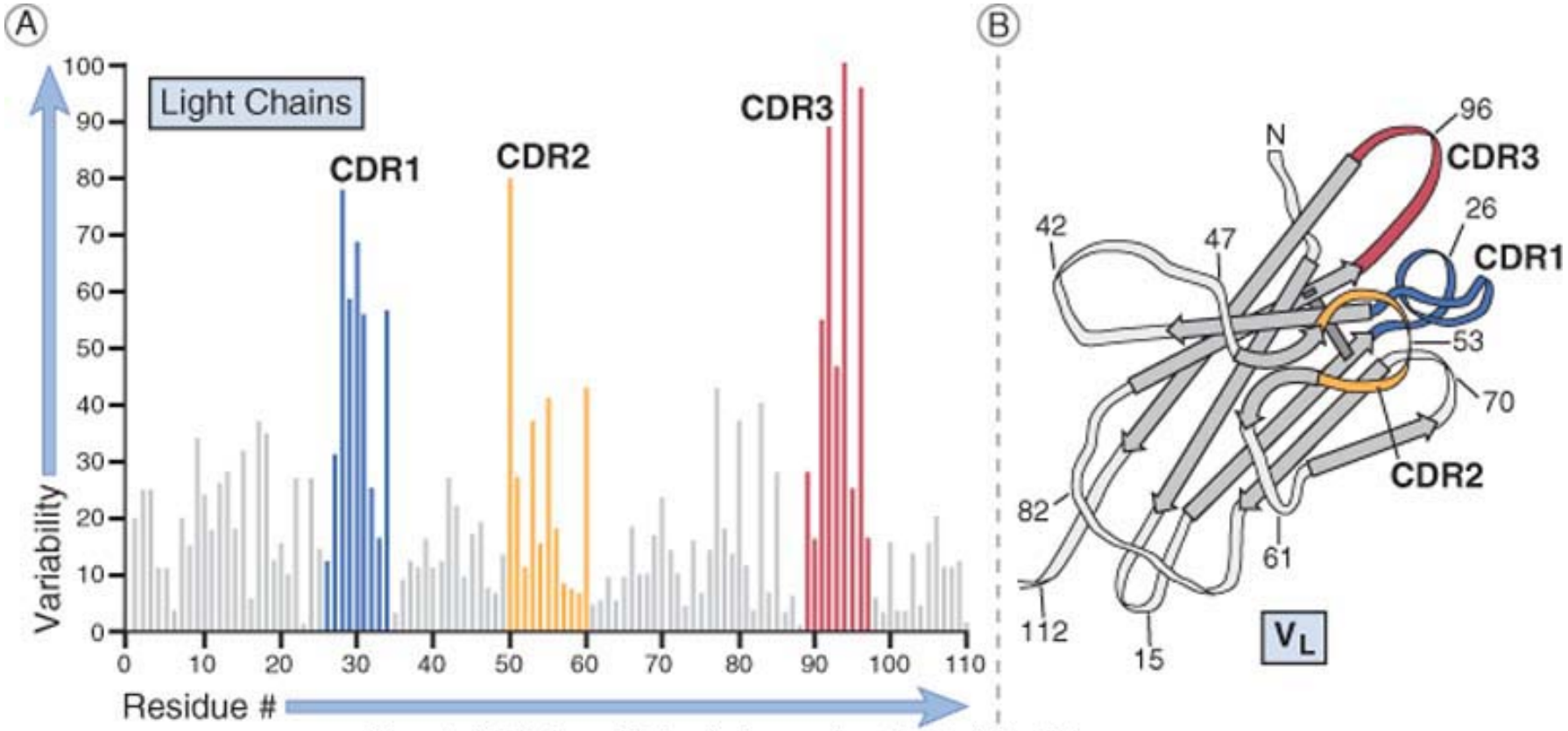
2 LIGHT CHAINS

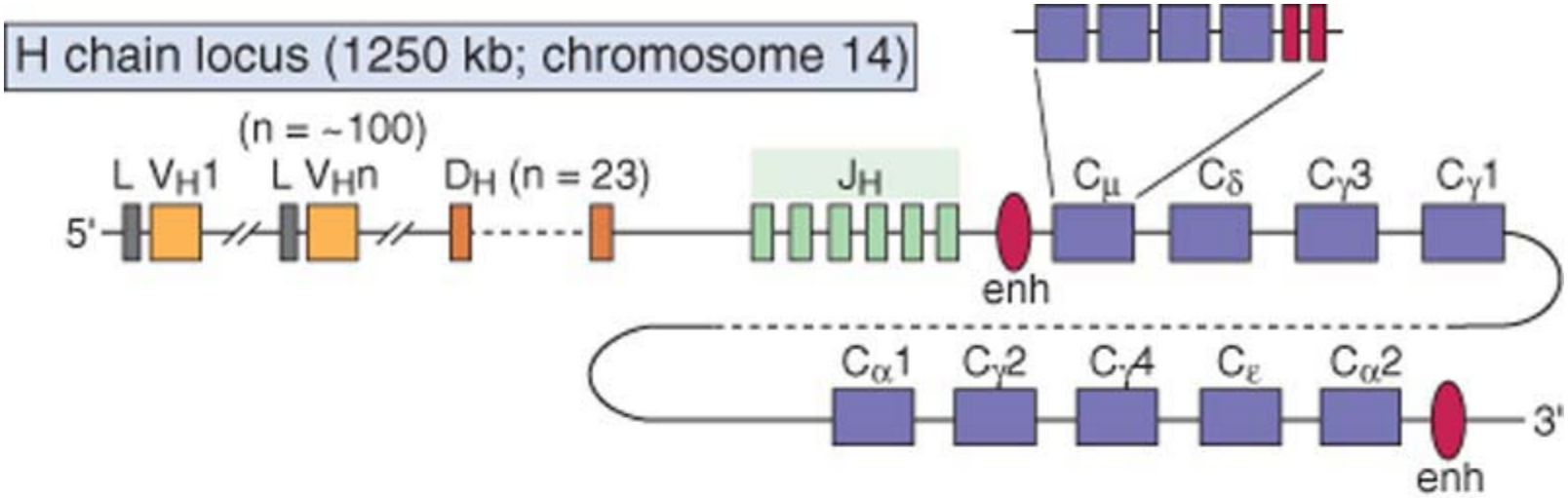
1 VARIABLE DOMAIN (VL)

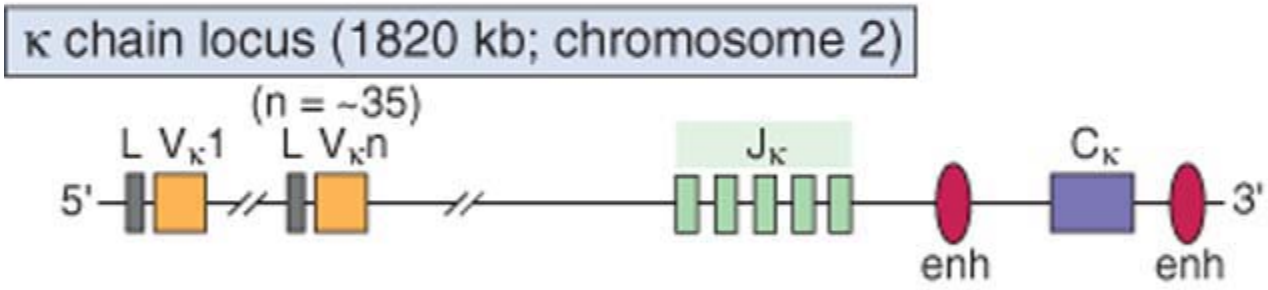
1 CONSTANT DOMAINS (CL)

(k or λ)

Aminoacid variability in antibody sequence




H chain locus (1250 kb; chromosome 14)
(n = ~100)
L VH1
L VHn
DH (n = 23)
JH
Cμ
Cδ
Cγ3
Cγ1
5'
enh
Cα1
Cγ2
Cγ4
Cε
Cα2
3'
enh


κ chain locus (1820 kb; chromosome 2)
(n = ~35)
L Vκ1
L Vκn
Jκ
Cκ
5'
3'
enh
enh

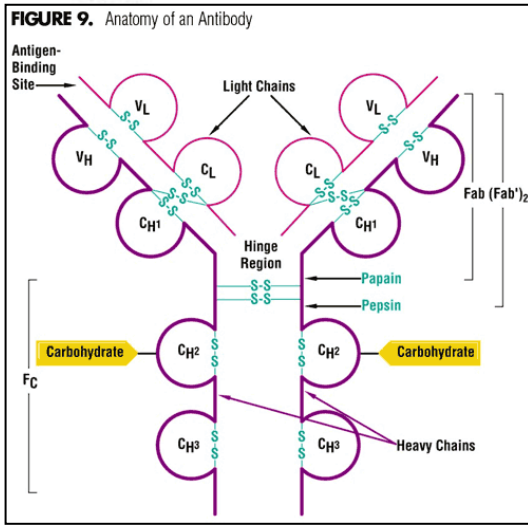
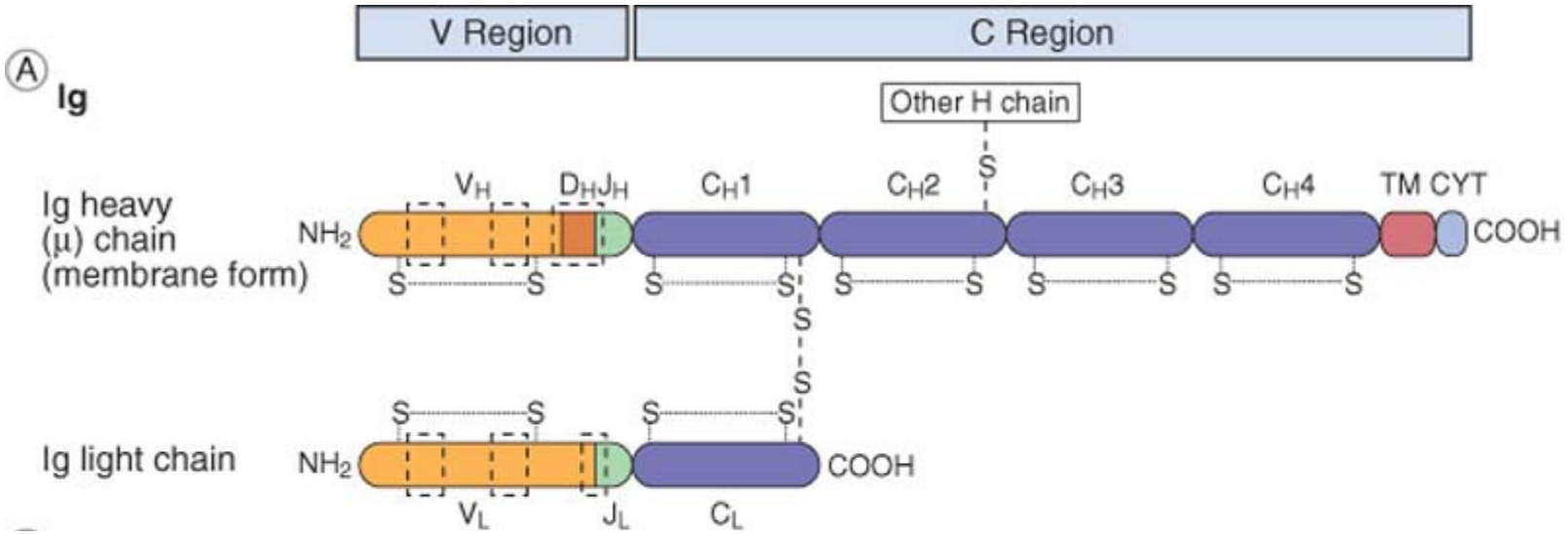
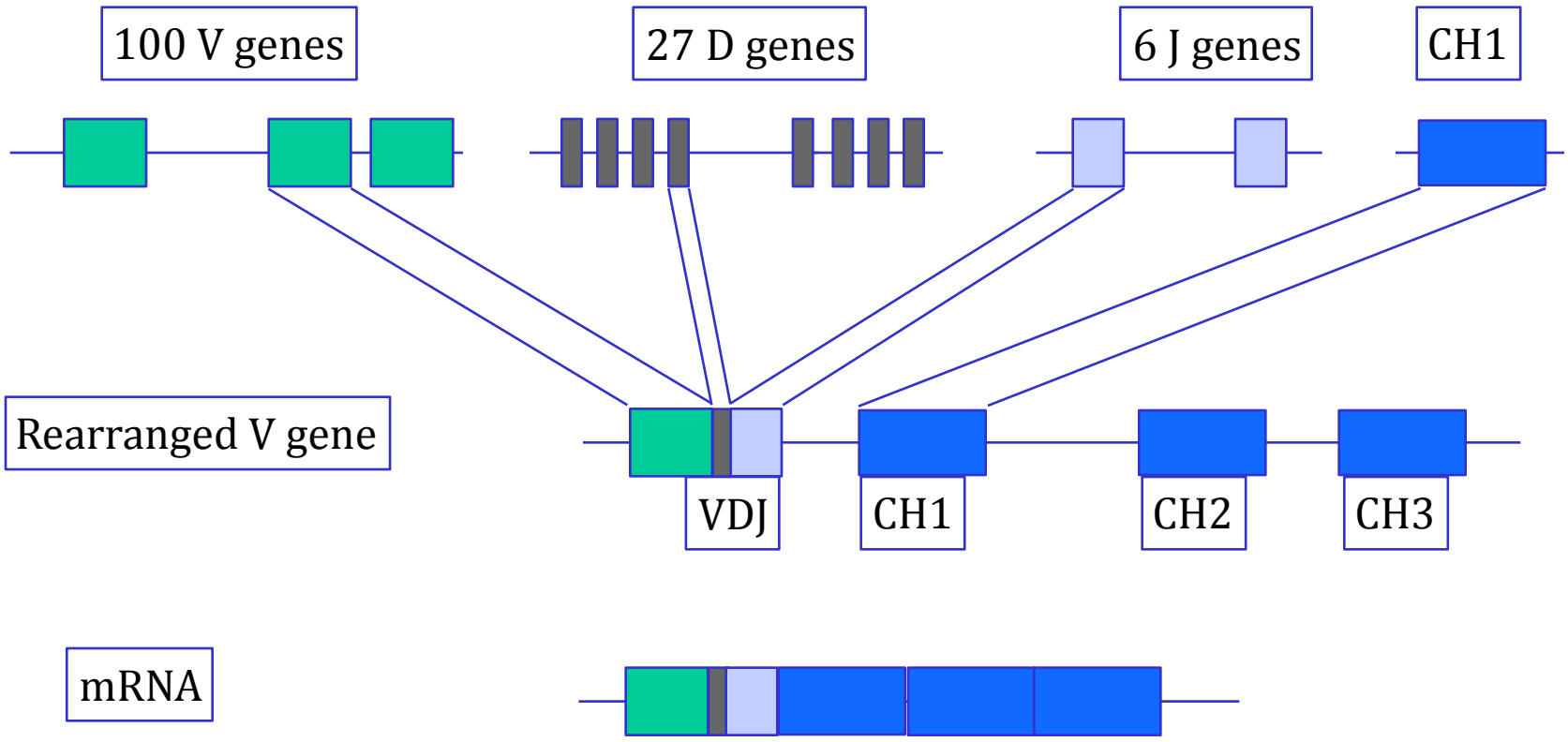


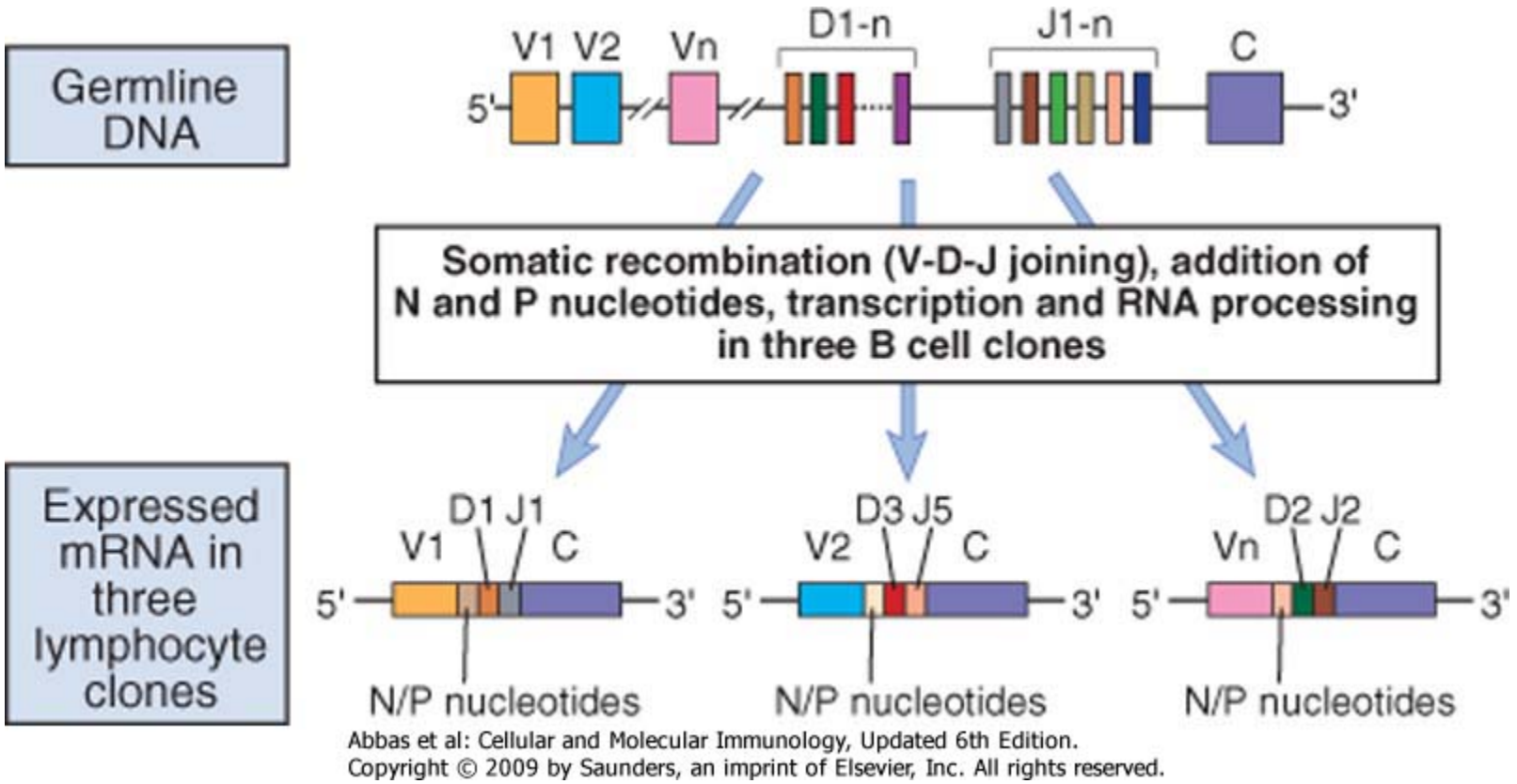
FIGURE 9. Anatomy of an Antibody
Antigen-Binding Site
Light Chains
VL
VH
CL
CH1
Hinge Region
Papain
Pepsin
Fab (Fab')2
Carbohydrate
CH2
Fc
CH3
Heavy Chains


A
Ig
V Region
C Region
Other H chain
Ig heavy
(μ) chain
(membrane form)
NH2
VH
DHJH
CH1
CH2
CH3
CH4
TM CYT
COOH
S
Ig light chain
NH2
COOH
VL
JL
CL

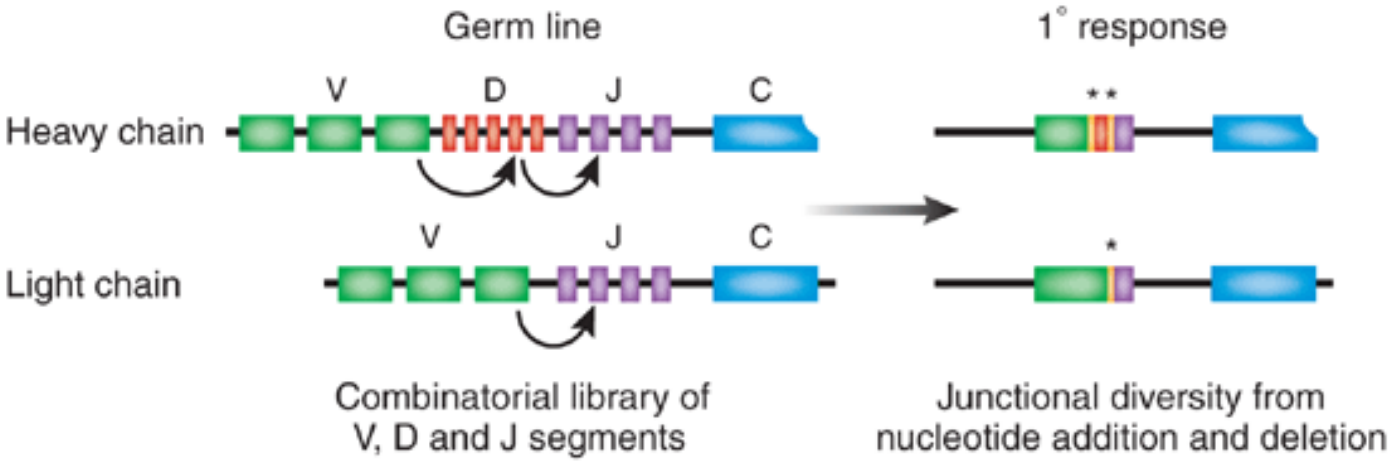
How VH genes are made and used in vivo



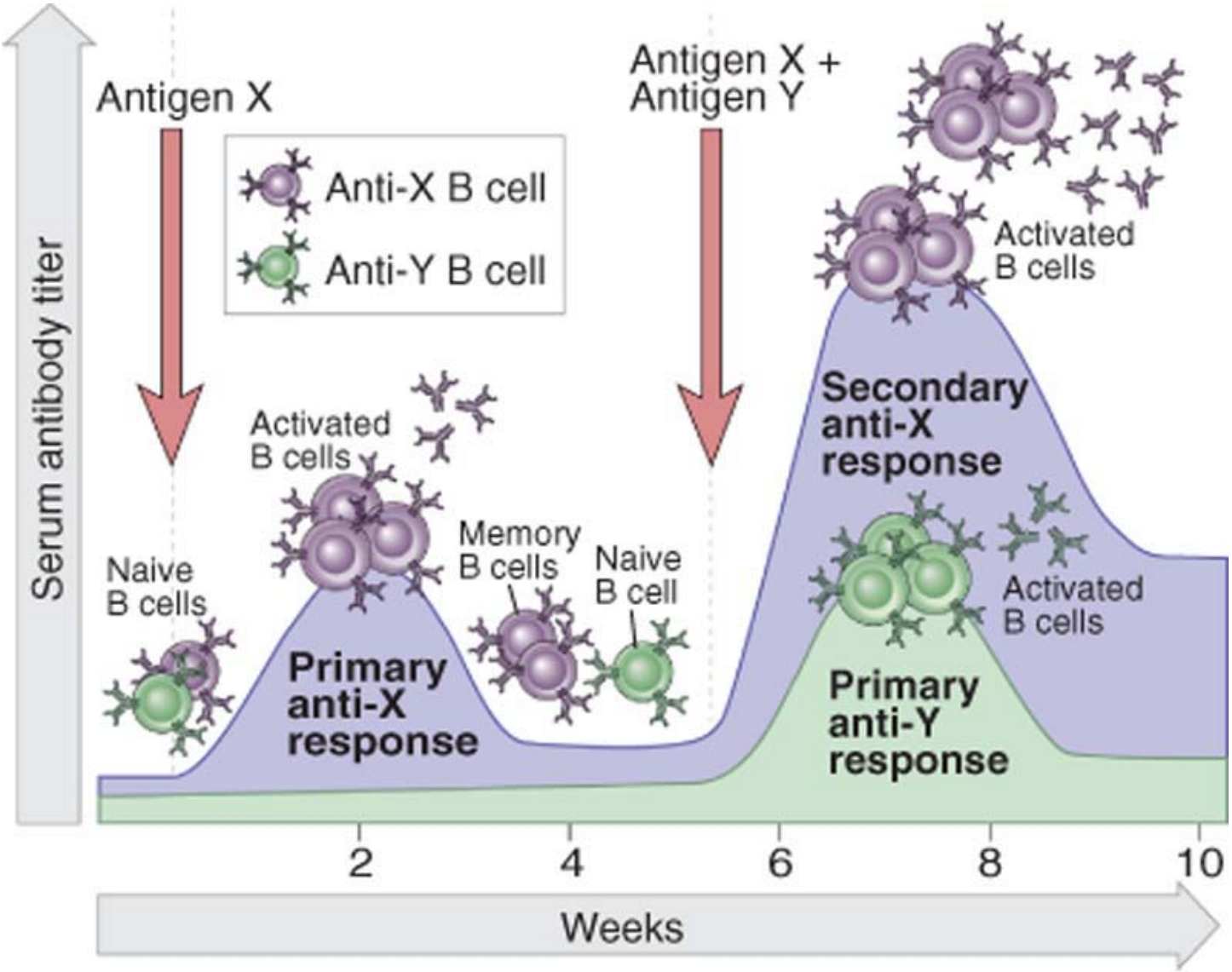
Math: 100 x 27 x 6= 16200 possible V_H



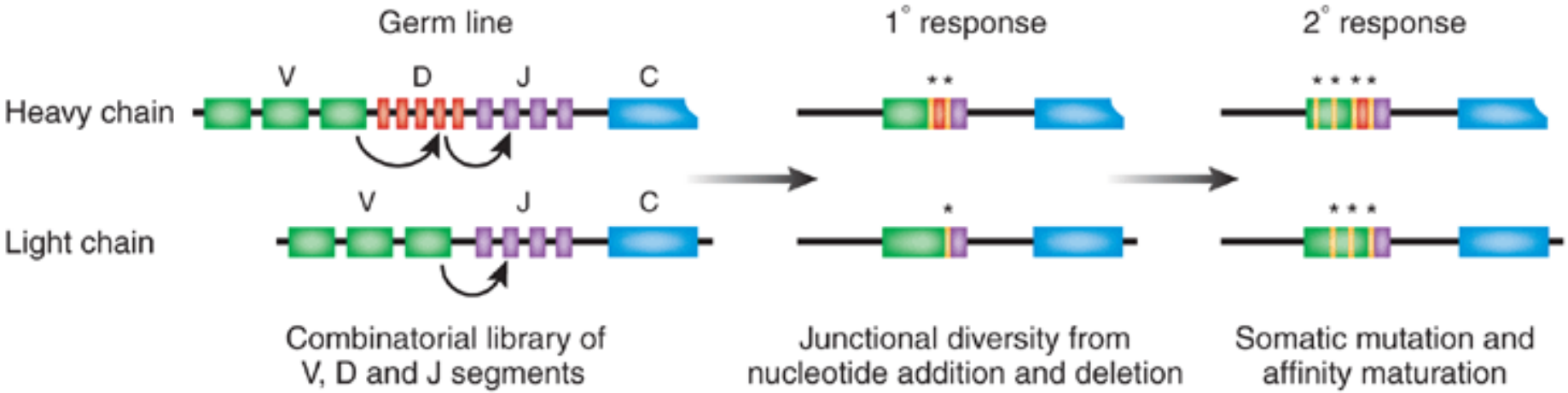
How are antibody genes rearranged in vivo?

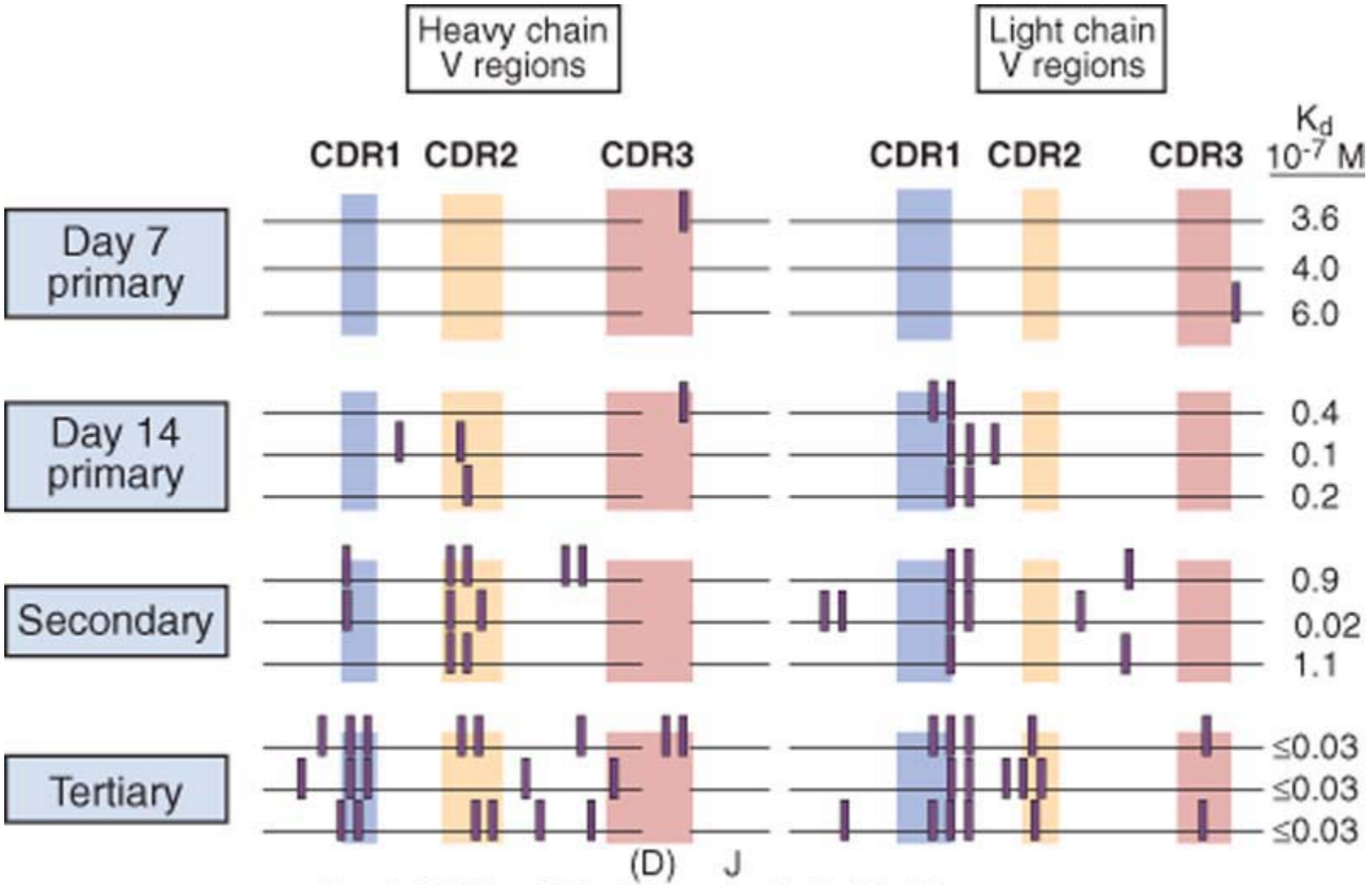


Timing for antibody production



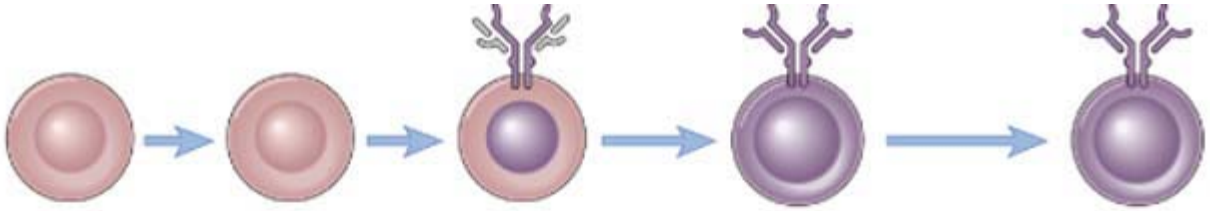
How are antibody genes rearranged in vivo?





Final variability of antibodies: $>10^9$

Where?



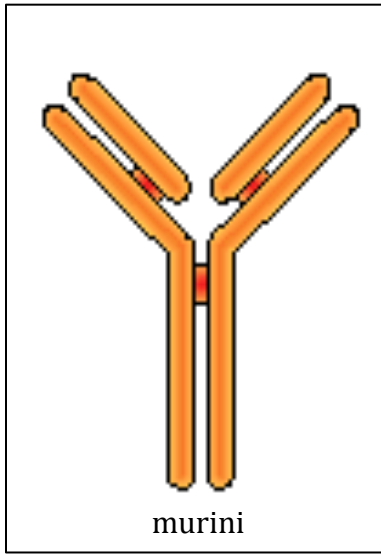
Stage of maturation	Stem cell	Pro-B	Pre-B	Immature B	Mature B
Proliferation					
Rag expression					
TdT expression					
Ig DNA, RNA	Unrecombined (germline) DNA	Unrecombined (germline) DNA	Recombined H chain gene (VDJ); μ mRNA	Recombined H chain gene (VDJ), κ or λ genes (VJ); μ or κ or λ mRNA	Alternative splicing of VDJ-C RNA (primary transcript), to form C_μ and C_δ mRNA
Ig expression	None	None	Cytoplasmic μ and pre-B receptor–associated μ	Membrane IgM (μ+ κ or λ light chain)	Membrane IgM and IgD
Surface markers	$CD43^+$	$CD43^+$ $CD19^+$ $CD10^+$	$B220^{lo}$ $CD43^+$	IgM^{lo} $CD43^-$	IgD^+ IgM^+ $CD23^+$
Anatomic site	Bone marrow				Periphery
Response to antigen	None	None	None	Negative selection (deletion), receptor editing	Activation (proliferation and differentiation)

Monoclonal antibodies

Murine monoclonal antibodies produced by hybridomas

Human immune response to mouse antibodies

HAMA = Human Anti-Mouse Antibodies


murini

- Endanger the patient
- enhance the clearance of the Ab
- reduce its therapeutic effect.

Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6851–6855, November 1984
Immunology

Chimeric human antibody molecules: Mouse antigen-binding domains with human constant region domains

(transfection/protoplast fusion/calcium phosphate transfection/intronic controlling elements/transfectoma)

SHERIE L. MORRISON*, M. JACQUELINE JOHNSON†, LEONARD A. HERZENBERG†, AND VERNON T. OI‡

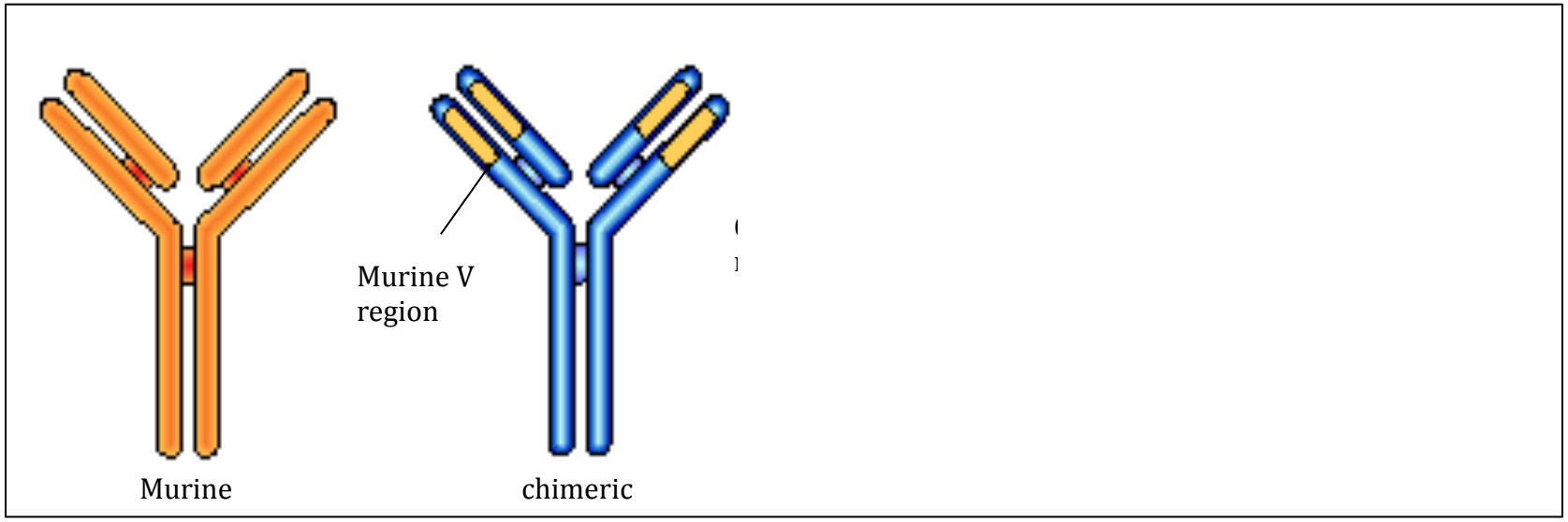
*Department of Microbiology and the Cancer Center, Institute for Cancer Research, College of Physicians and Surgeons, Columbia University, New York, NY 10032; †Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305; and ‡Becton–Dickinson Monoclonal Center, 2375 Garcia Avenue, Mountain View, CA 94043

Contributed by Leonard A. Herzenberg, August 1, 1984

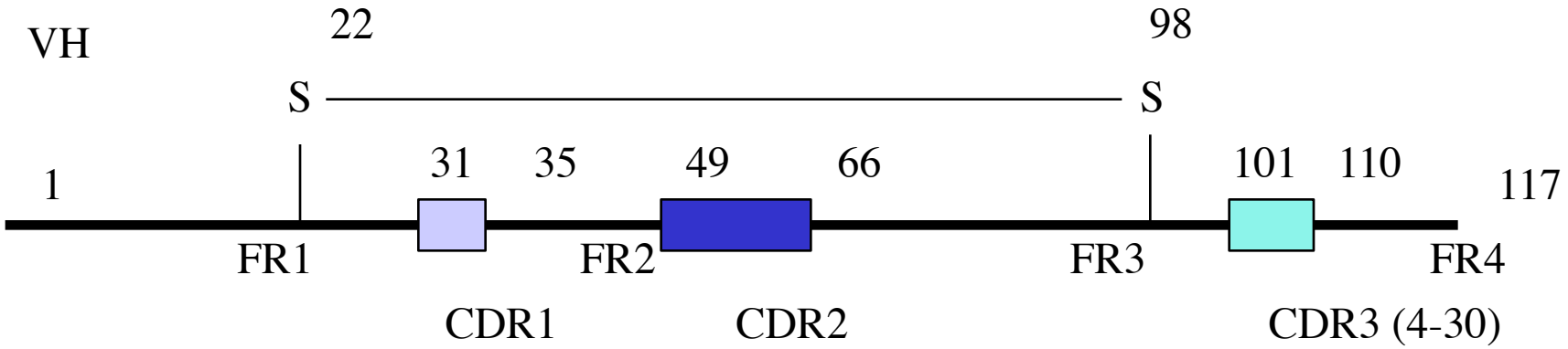
Monoclonal antibodies

Murine monoclonal antibodies produced by hybridomas

Chimeric recombinant antibodies: 70% human DNA



Rearranged V gene structure



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Display Settings: Abstract | Send to:

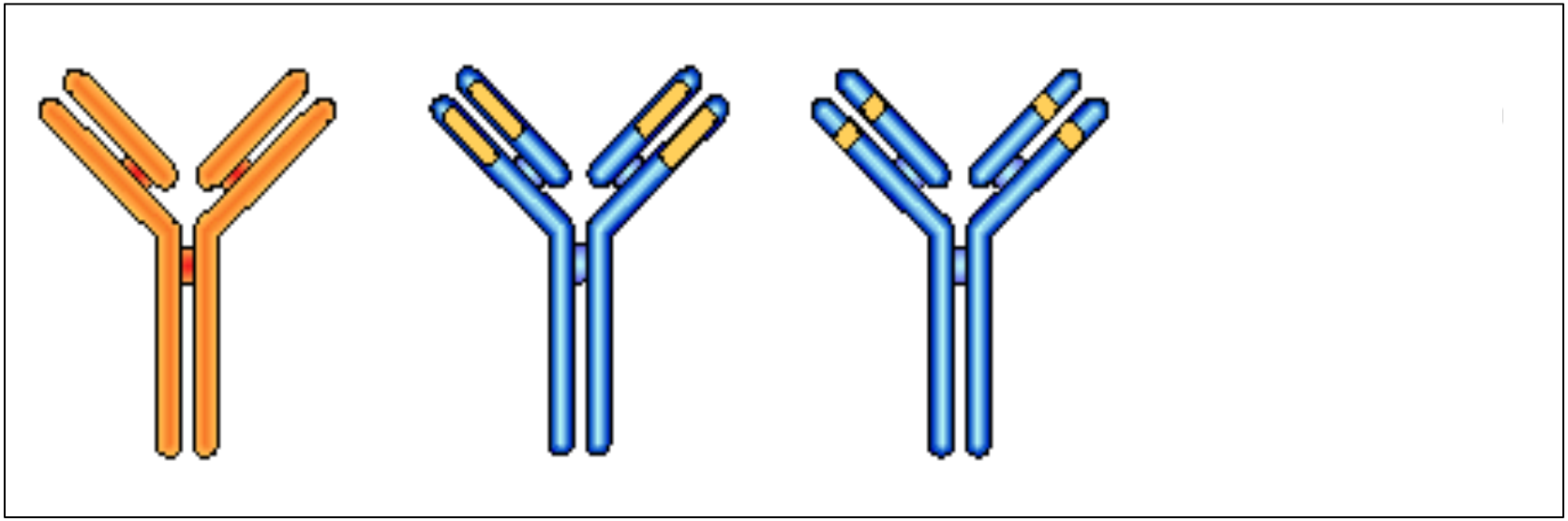
Nature. 1986 May 29-Jun 4;321(6069):522-5.

Replacing the complementarity-determining regions in a human antibody with those from a mouse.

Jones PT, Dear PH, Foote J, Neuberger MS, Winter G.

Abstract

The variable domains of an antibody consist of a beta-sheet framework with hypervariable regions (or complementarity-determining regions--CDRs) which fashion the antigen-binding site. Here we attempted to determine whether the antigen-binding site could be transplanted from one framework to another by grafting the CDRs. We substituted the CDRs from the heavy-chain variable region of mouse antibody B1-8, which binds the hapten NP-cap (4-hydroxy-3-nitrophenacetyl caproic acid; KNP-cap = 1.2 microM), for the corresponding CDRs of a human myeloma protein. We report that in combination with the B1-8 mouse light chain, the new antibody has acquired the hapten affinity of the B1-8 antibody (KNP-cap = 1.9 microM). Such 'CDR replacement' may offer a means of constructing human monoclonal antibodies from the corresponding mouse monoclonal antibodies.



- Humanized antibodies (95% human DNA umano; only CDRs are murine)

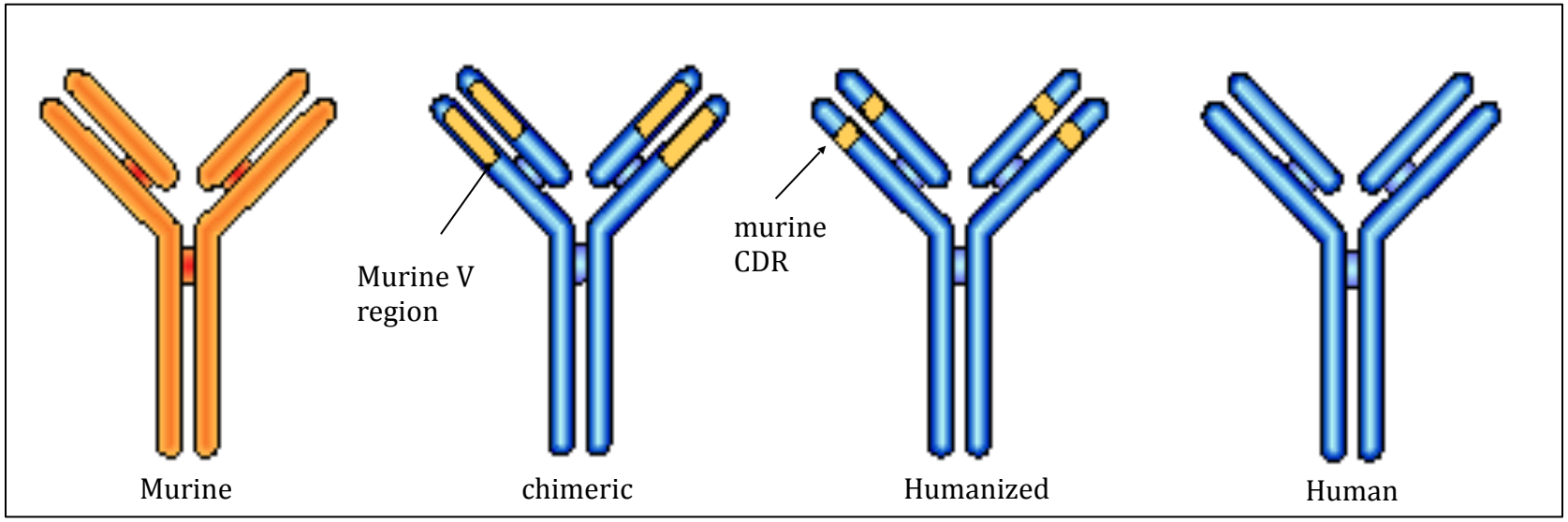
Monoclonal antibodies

Murine monoclonal antibodies produced by hybridomas

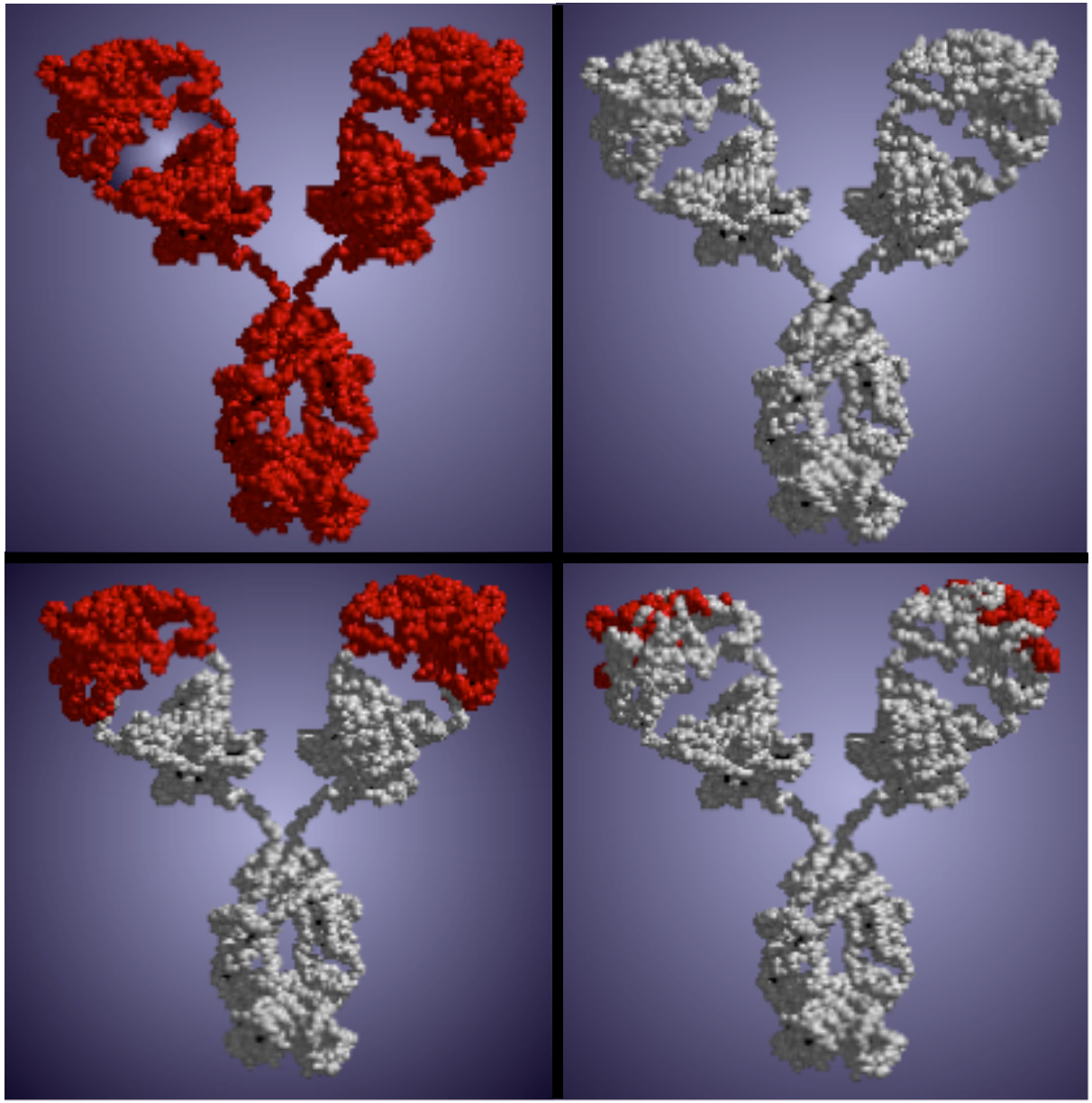
Chimeric recombinant antibodies: 70% human DNA

Humanized recombinant antibodies: 95% human DNA

Human antibodies: 100% human DNA



What they really look like



Mouse

Human

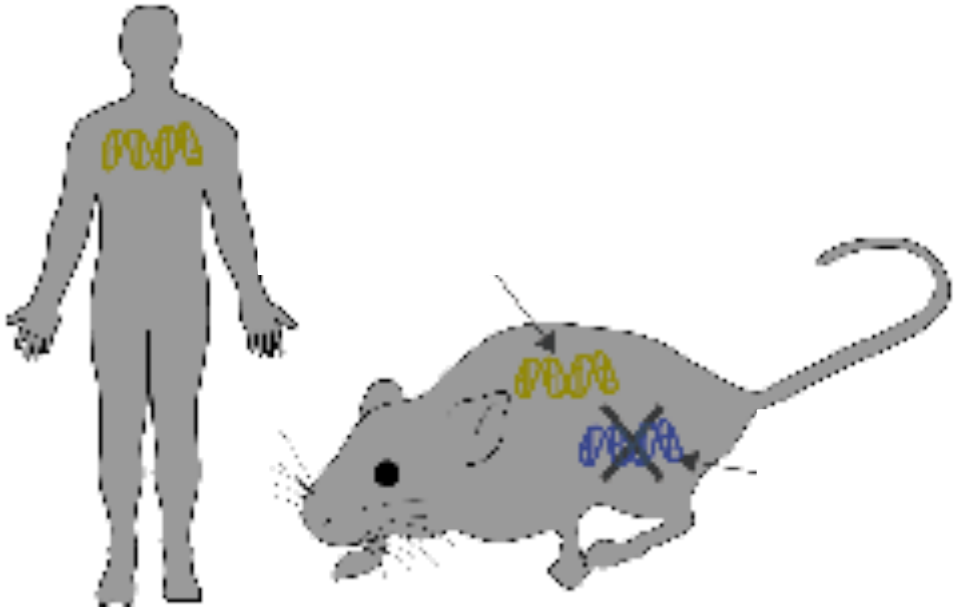
Chimeric

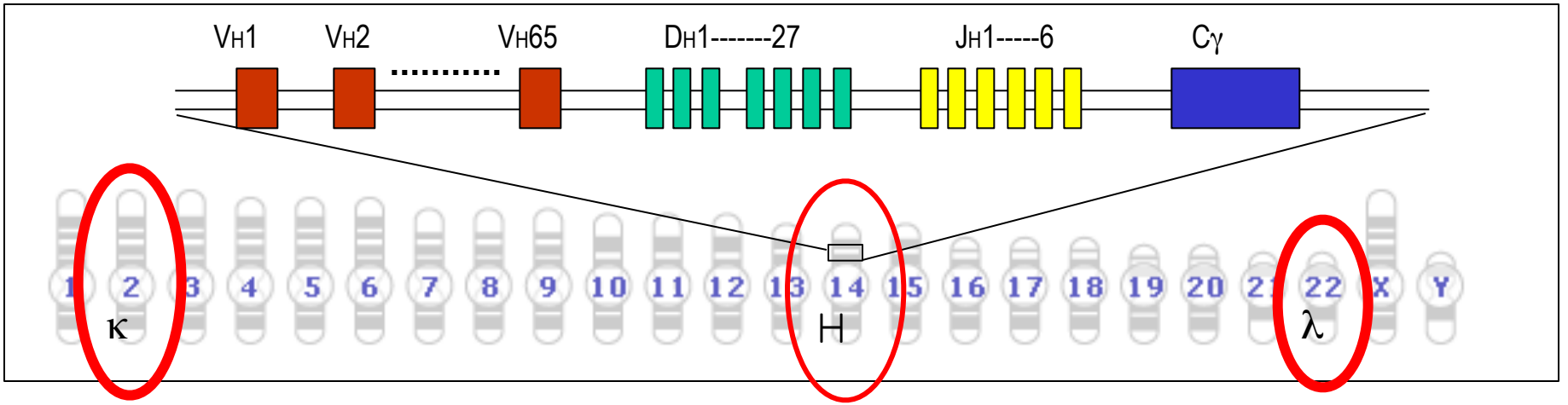
Humanized

Making human antibodies

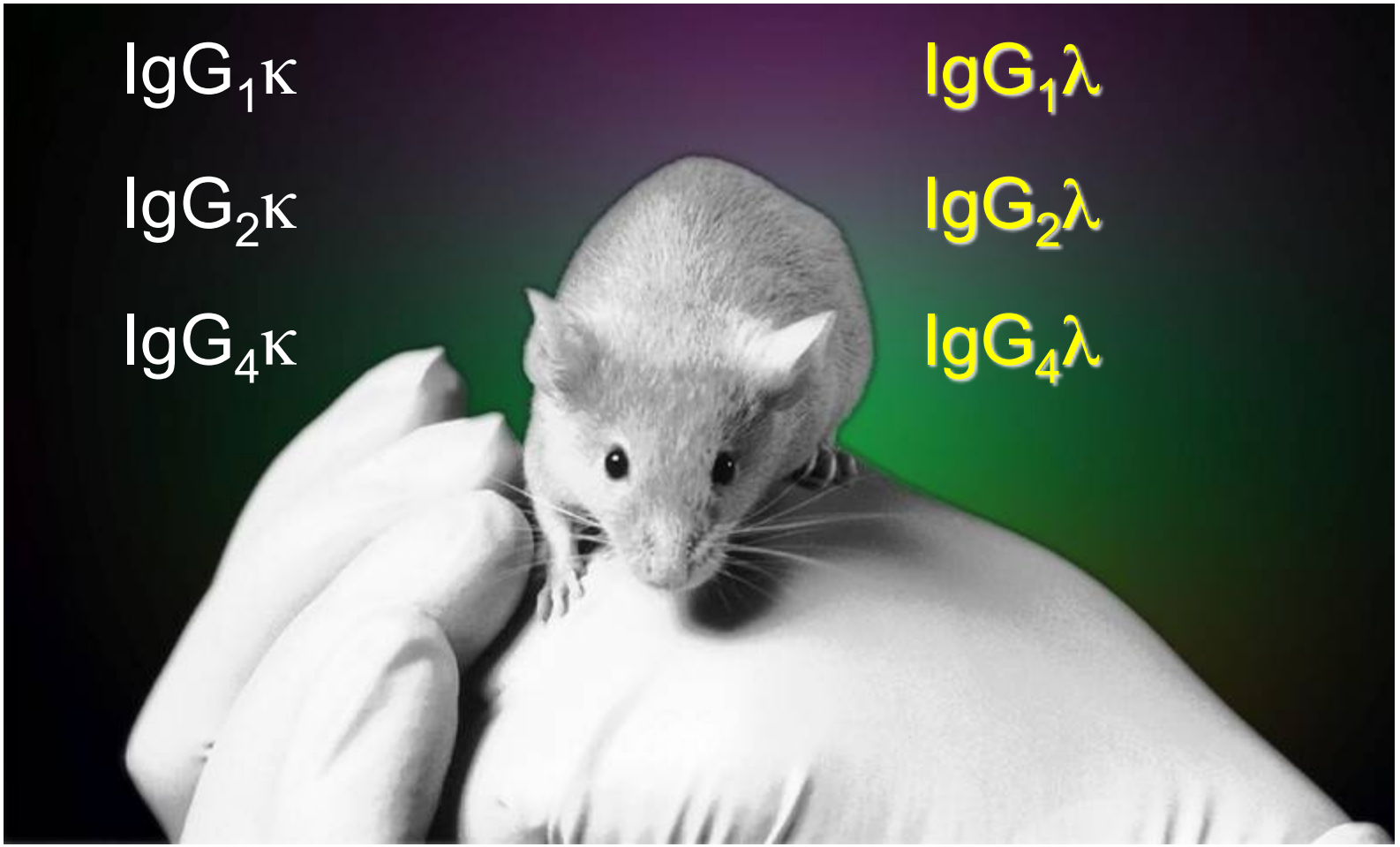
- Transgenic animals
- Phage/microbial/yeast display

Transgenic Mouse





XenoMouse®-κλ Strains



Nature Biotechnology **25**, 1134 - 1143 (2007)
Published online: 5 October 2007 | doi:10.1038/nbt1337

From XenoMouse technology to panitumumab, the first fully human antibody product from transgenic mice

Aya Jakobovits[1], Rafael G Amado[2], Xiaodong Yang[3], Lorin Roskos[4] & Gisela Schwab[5]

Therapeutic monoclonal antibodies have shown limited efficacy and safety owing to immunogenicity of mouse sequences in humans. Among the approaches developed to overcome these hurdles were transgenic mice genetically engineered with a 'humanized' humoral immune system. One such transgenic system, the XenoMouse, has succeeded in recapitulating the human antibody response in mice, by introducing nearly the entire human immunoglobulin loci into the germ line of mice with inactivated mouse antibody machinery. XenoMouse strains have been used to generate numerous high-affinity, fully human antibodies to targets in multiple disease indications, many of which are progressing in clinical development. However, validation of the technology has awaited the recent regulatory approval of panitumumab (Vectibix), a fully human antibody directed against epidermal growth factor receptor (EGFR), as treatment for people with advanced colorectal cancer. The successful development of panitumumab represents a milestone for mice engineered with a human humoral immune system and their future applications.

Transgenic mice

- These produce human antibodies following immunization
- The creation of monoclonal antibodies is the same as normal mice using hybridoma technology
- Antibody production is still required after selection

Making human antibodies

- Transgenic animals
- Phage/microbial/yeast display

The Royal Swedish Academy of Sciences has decided to award the **Nobel Prize in Chemistry 2018** with one half to Frances H. Arnold "for the directed evolution of enzymes" and the other half **jointly to George P. Smith and Sir Gregory P. Winter "for the phage display of peptides and antibodies**."

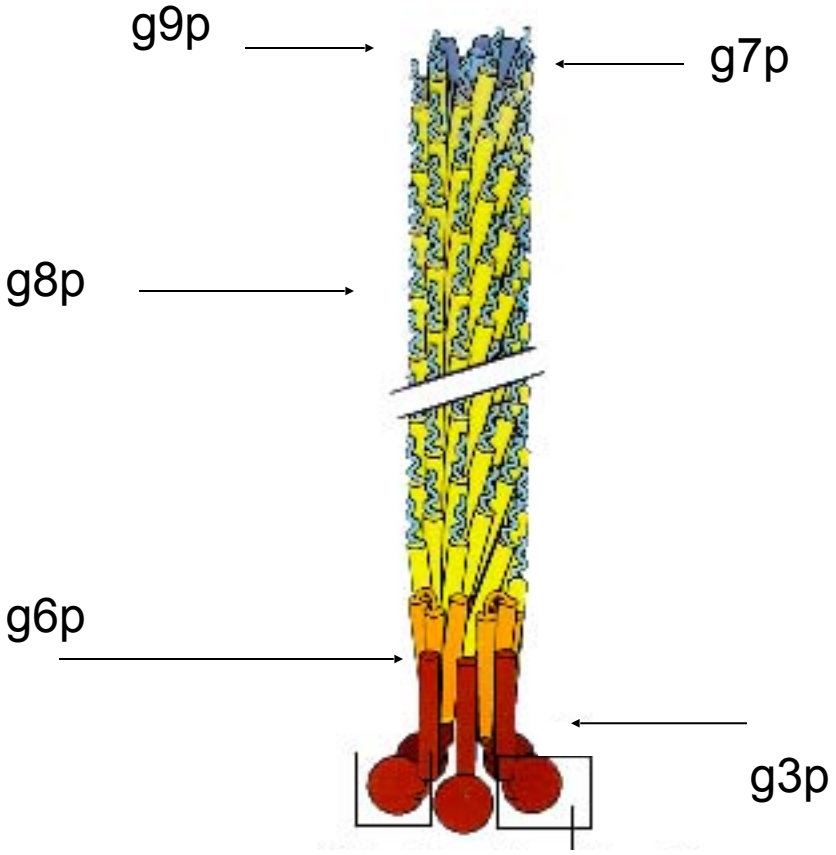
The power of evolution is revealed through the diversity of life. **The 2018 Nobel Laureates in Chemistry have taken control of evolution** and used it for purposes that bring the greatest benefit to humankind. Enzymes produced through directed evolution are used to manufacture everything from biofuels to pharmaceuticals. Antibodies evolved using a method called phage display can combat autoimmune diseases and, in some cases, cure metastatic cancer.

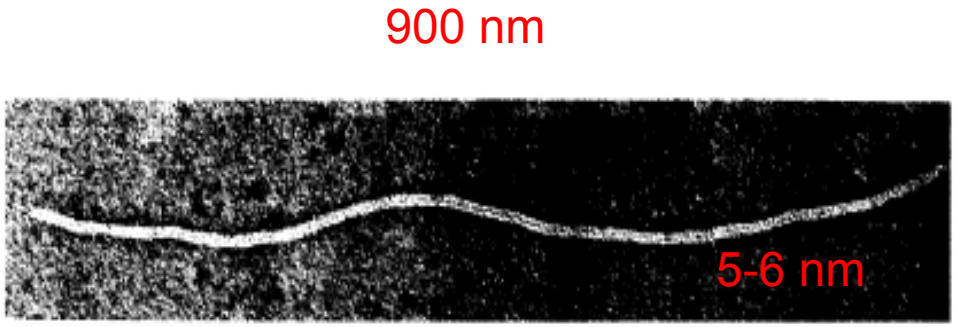
This year's Nobel Laureates have been inspired by the power of evolution and used the same principles – genetic change and selection – to develop proteins that solve humankind's chemical problems.

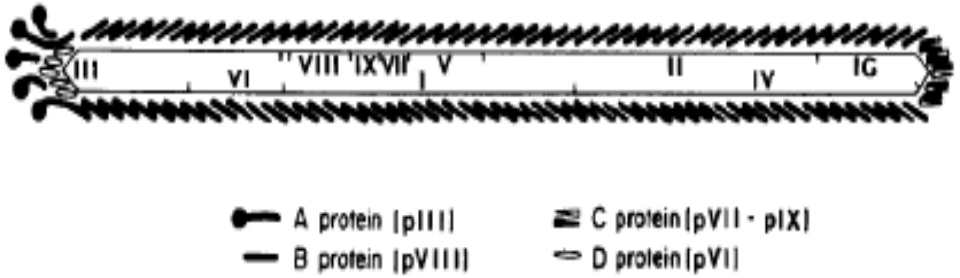
Antibody libraries

AIM: to allow the possibility to work with the antibody variability in the lab and not in the animal house

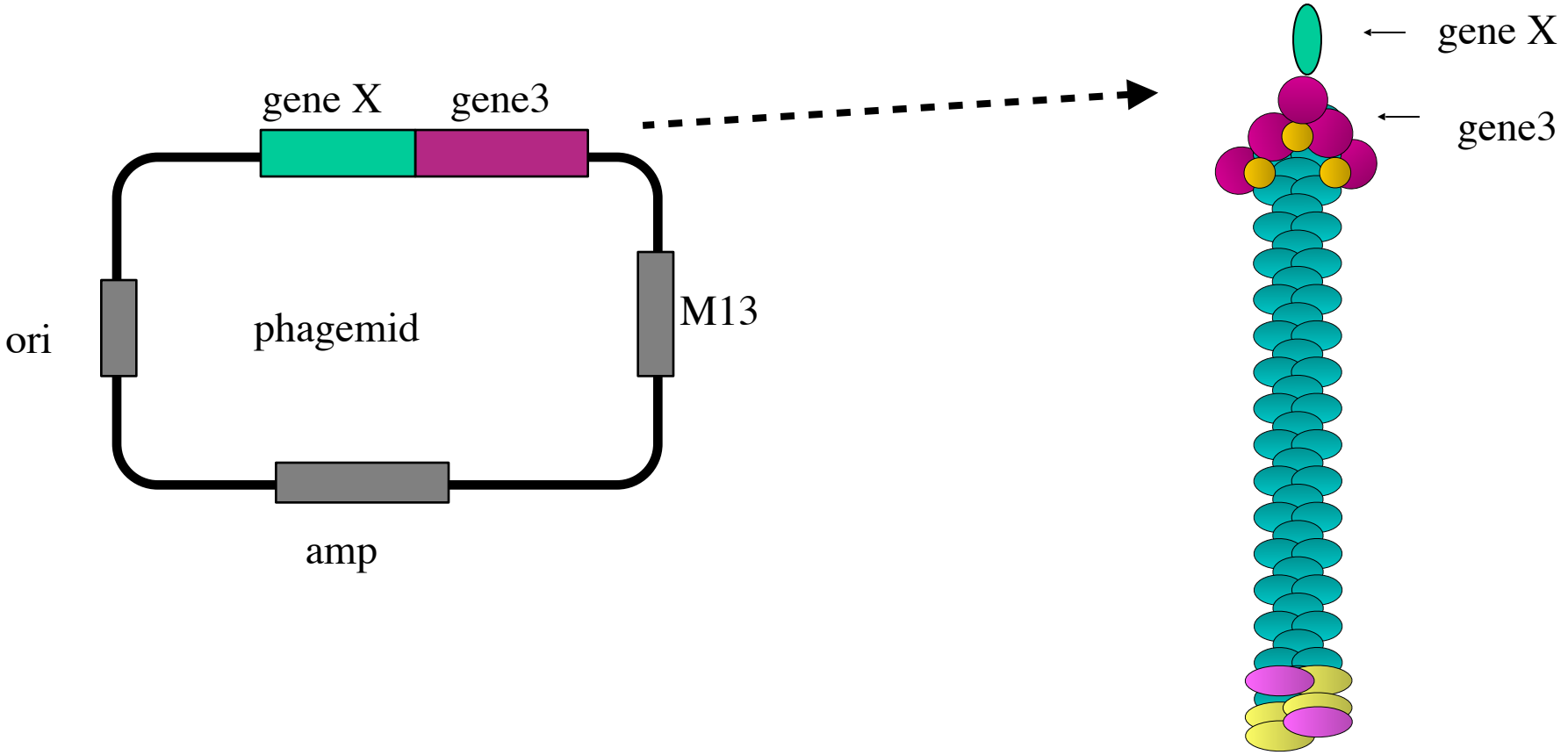
The biology of filamentous phages (f1)



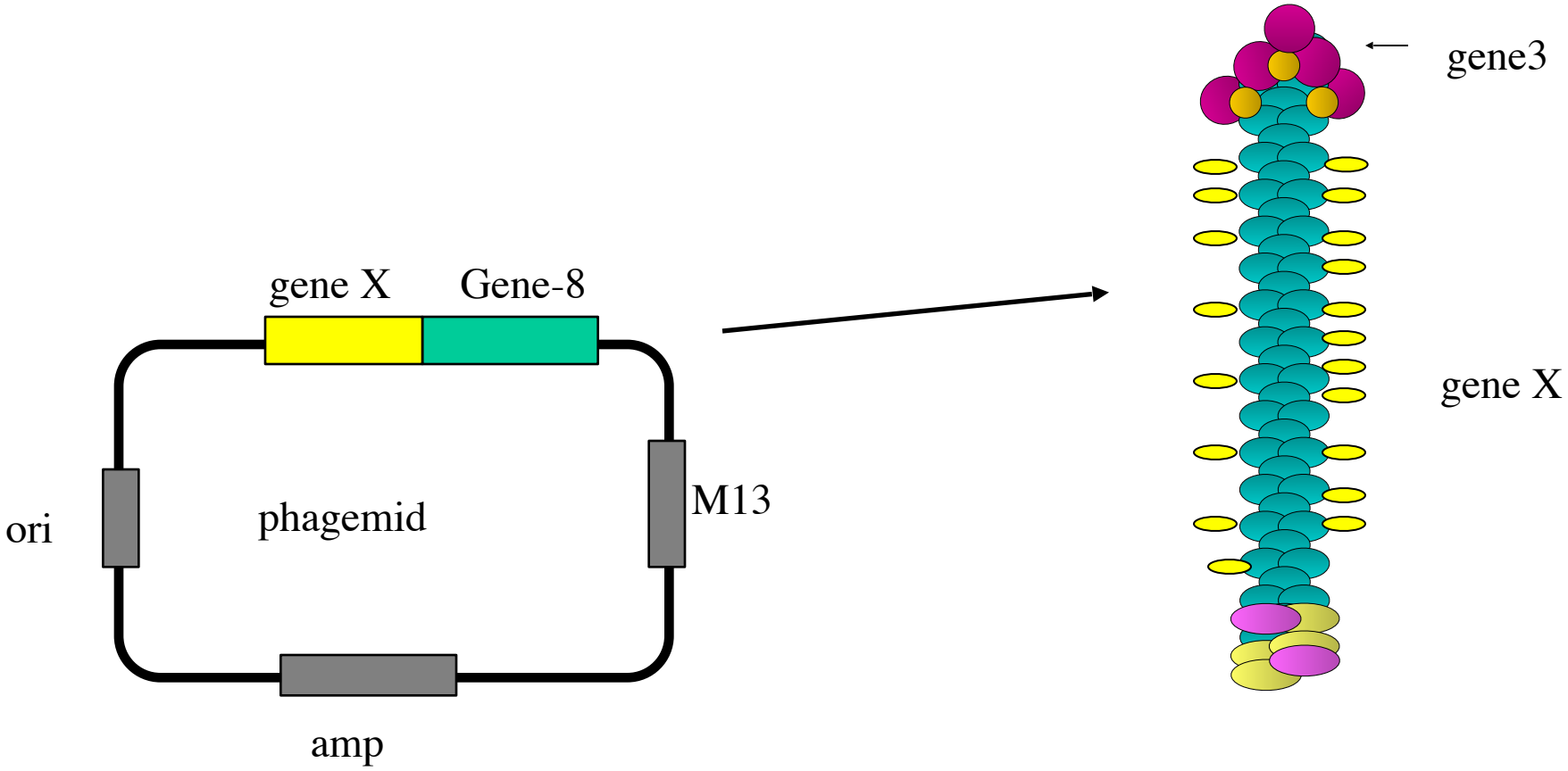




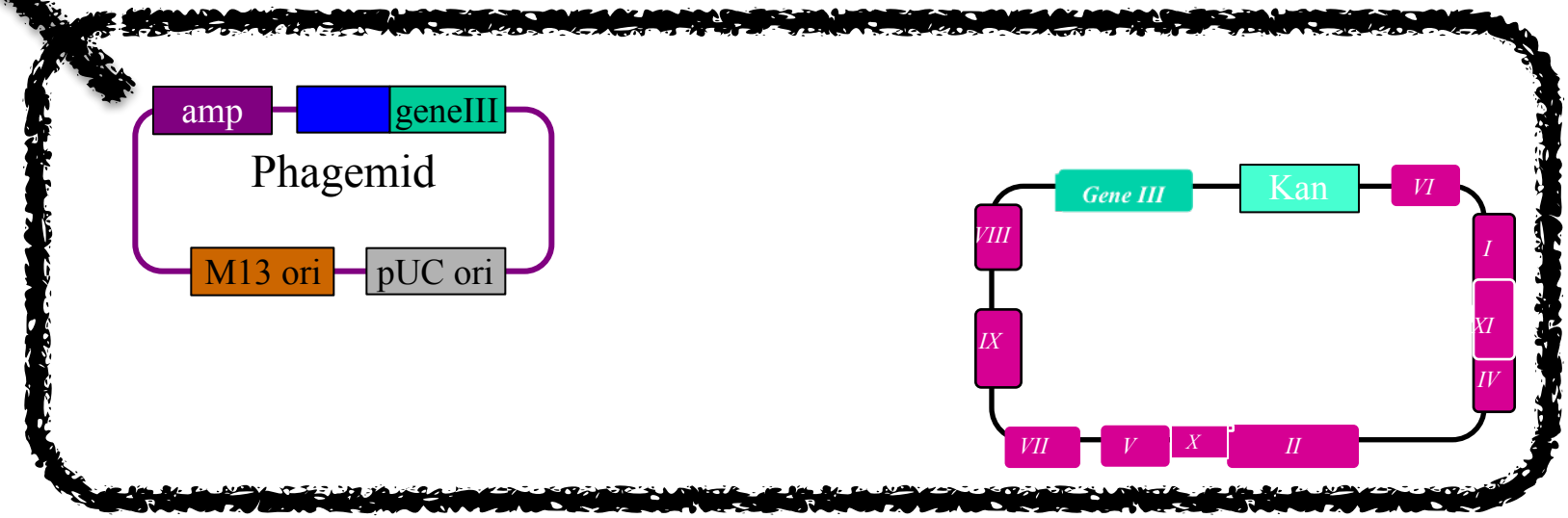
Phage Display Vector



Phage Display Vector

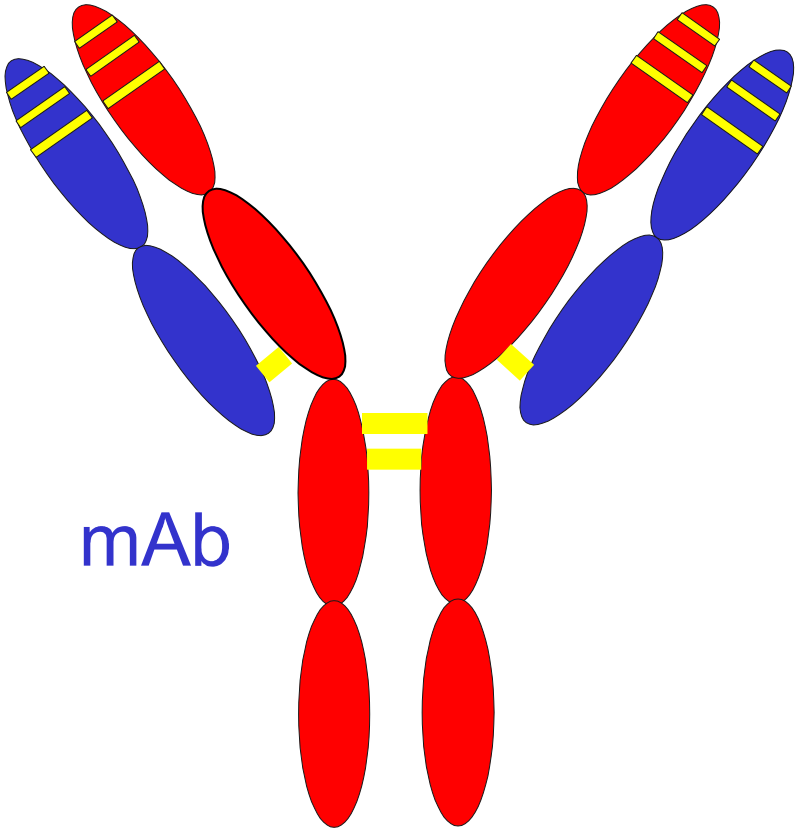


Phage production

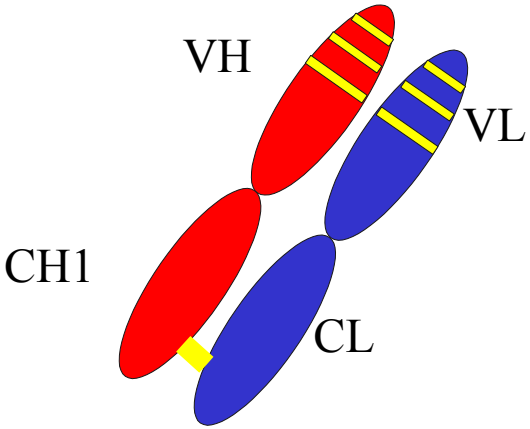


Infective (2. kan)

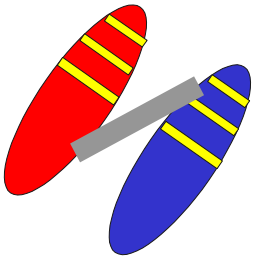
Infective (1. amp)



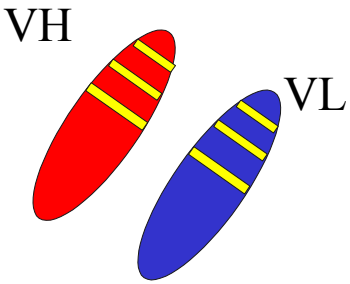
mAb



FAb



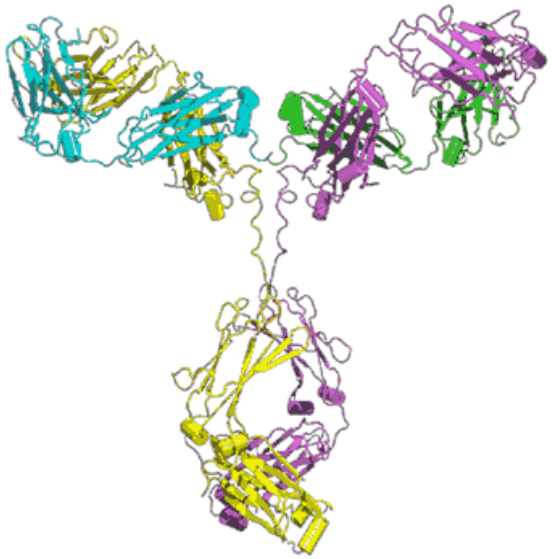
scFv



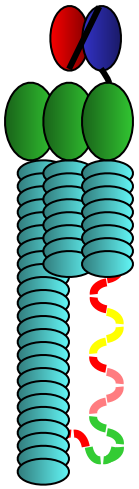
Fv

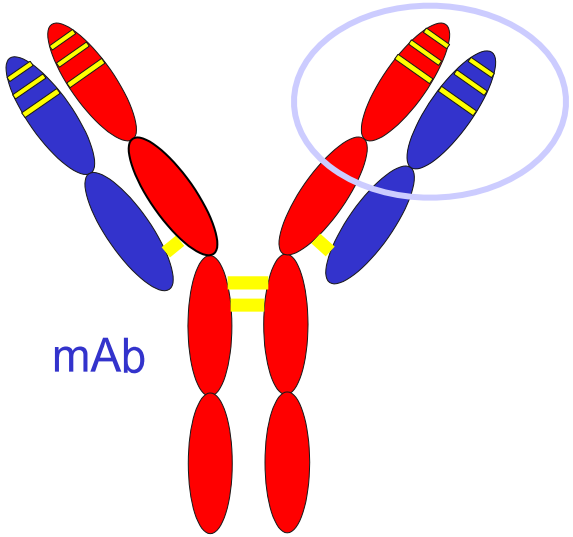
Single chain Fv fragment

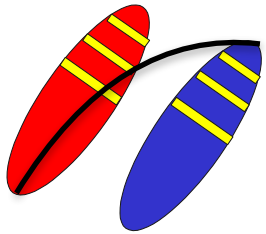
Antibody



Phage



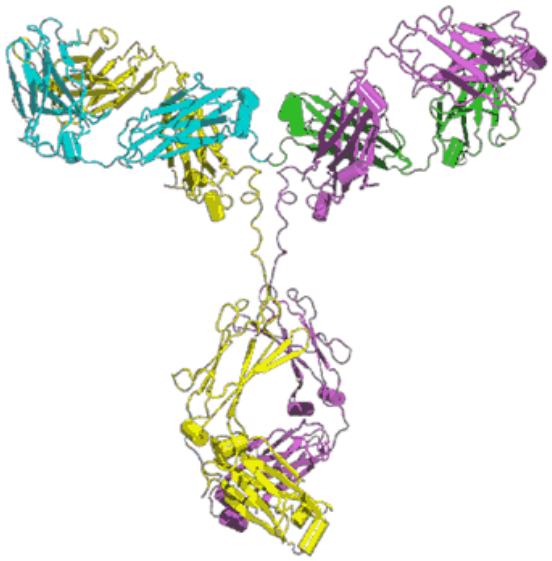


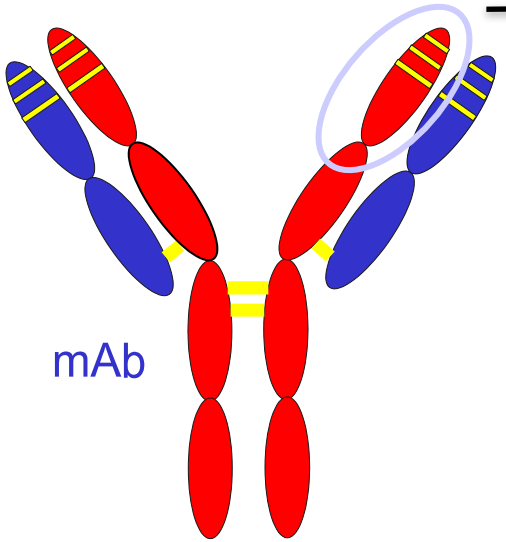


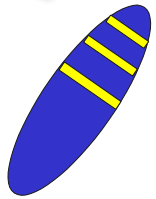
scFv

Antibody

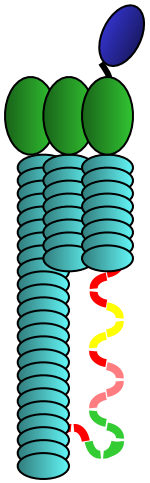
Phage



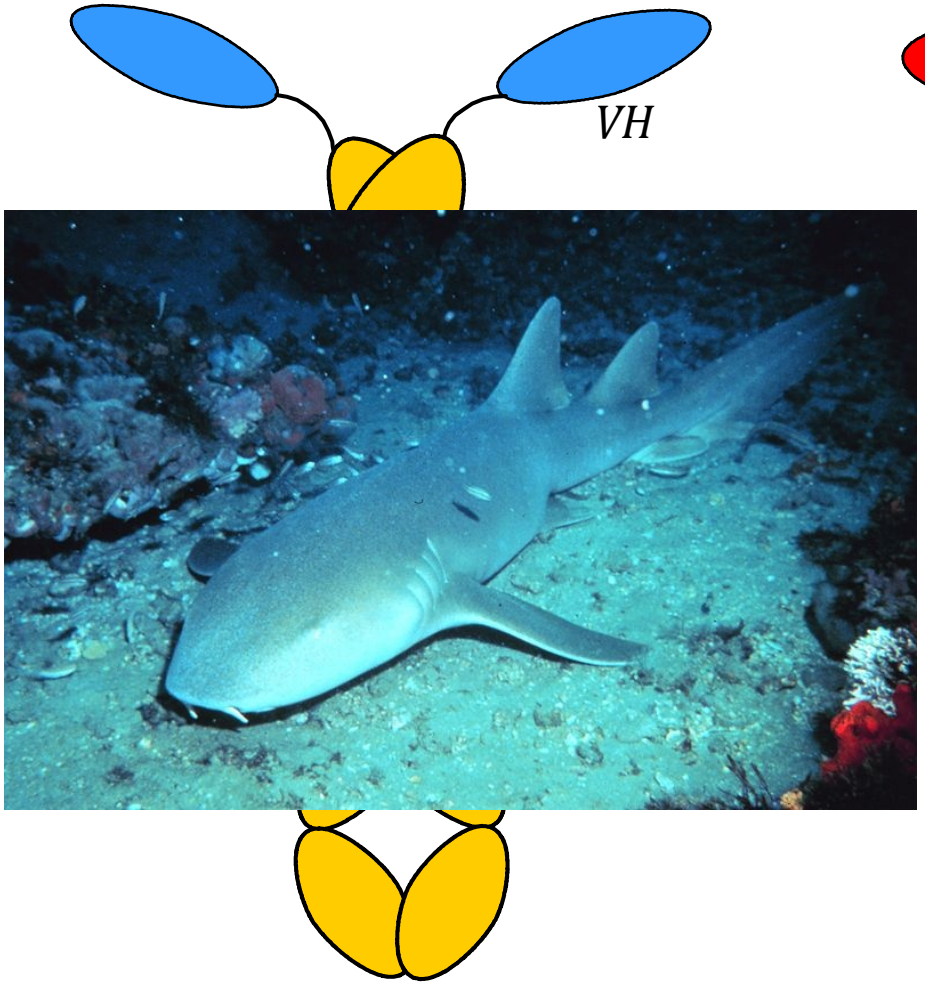




dAb



Some animals use natural dAbs



Shark

Camel lama

Points to consider when making and using antibody libraries

- The source of V region diversity (natural or synthetic)
- For natural V genes, the tissue source of V genes

V gene sources for making antibody libraries

- **Immune sources**: IgG V genes derived from PBL, or spleen from immunized animals or patients
  - High affinity antibodies against single antigens
- **Naive sources**: IgM/IgG V genes, derived from PBL, spleen, bone marrow
  - Antibodies against a wide diversity of antigens
- **Synthetic**: clone all or some V genes (human or mouse) and reconstitute CDRs for both VH and VL by PCR using oligonucleotides

Points to consider when making and using antibody libraries

- Antibody form (Single chain Fv (scFv) or Fab)
- The source of V region diversity (natural or synthetic)
- For natural V genes, the tissue source of V genes
- How to assemble the V regions
- Type of library
- The display protein used (p3, p8 or p9)
- **Selection strategy**

Selection

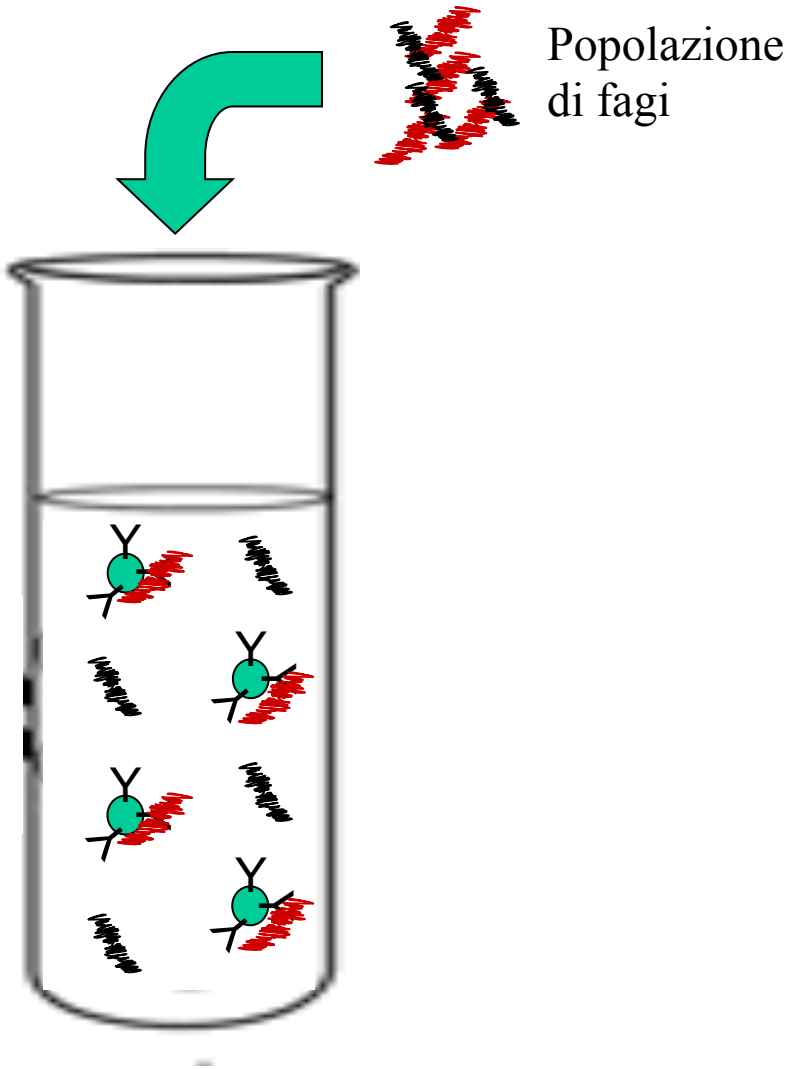
Sfere paramagnetiche

Antigene

Sfere attivate

Reactive phage

Non-reactive phage



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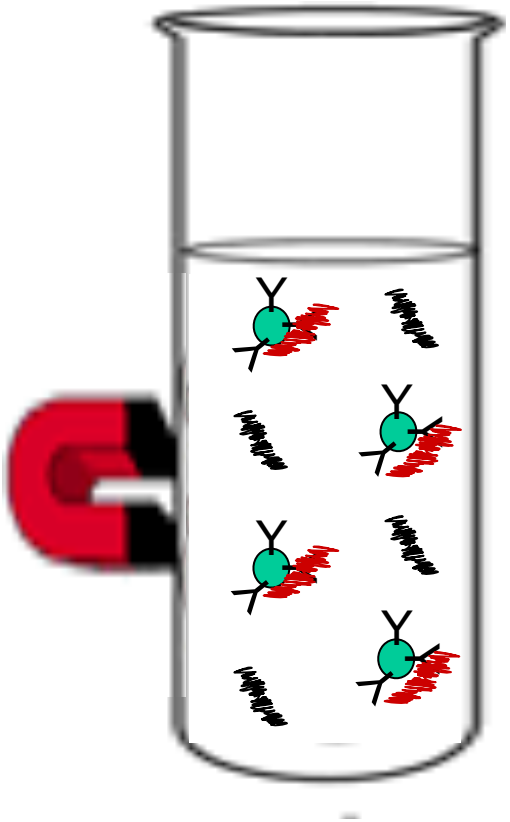

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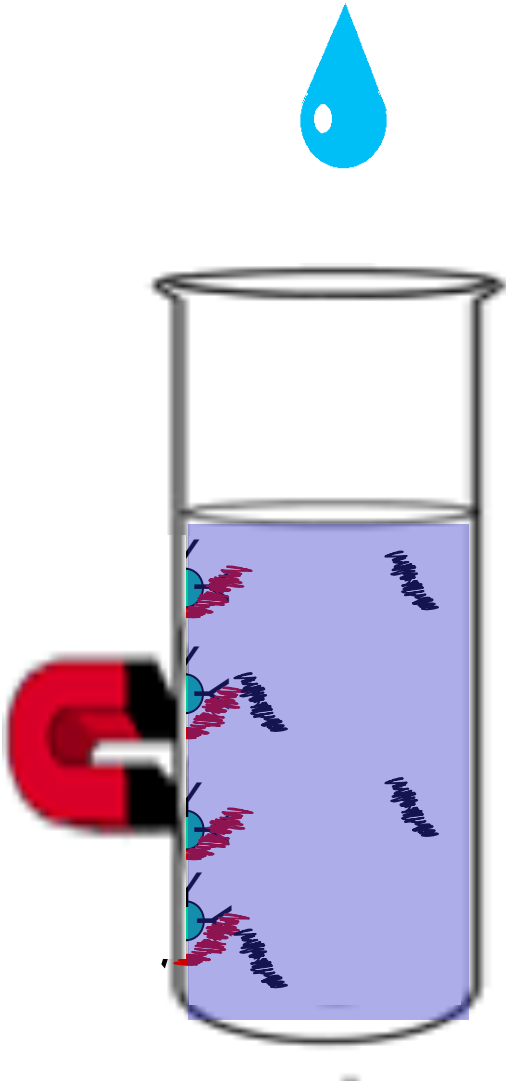


Selezione

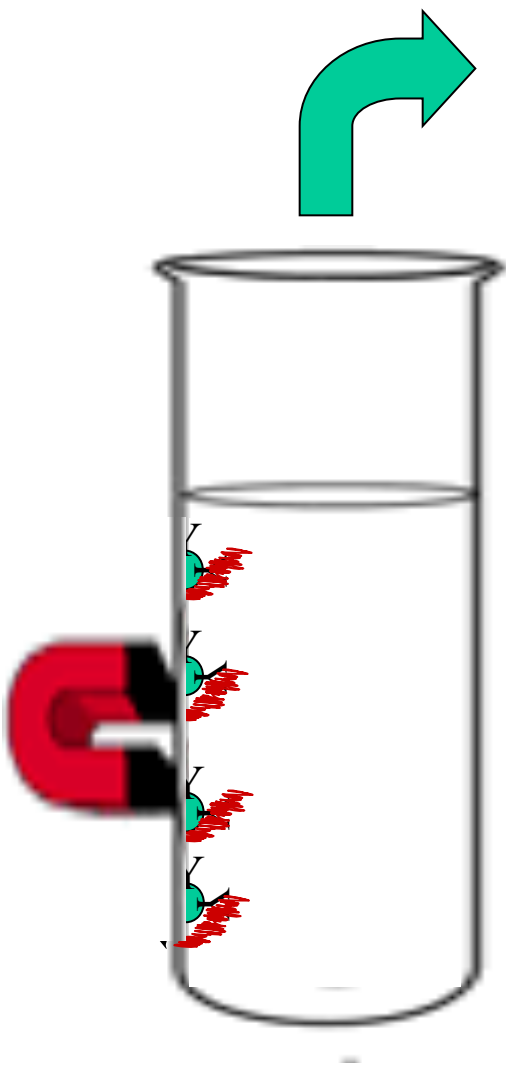
Sfere paramagnetiche

Anticorpi da pazienti

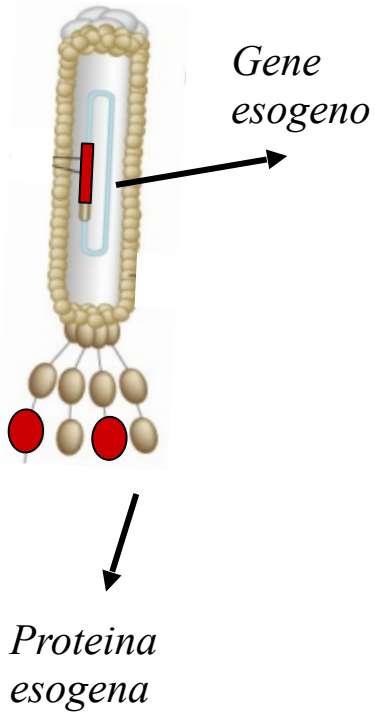
Sfere attivate

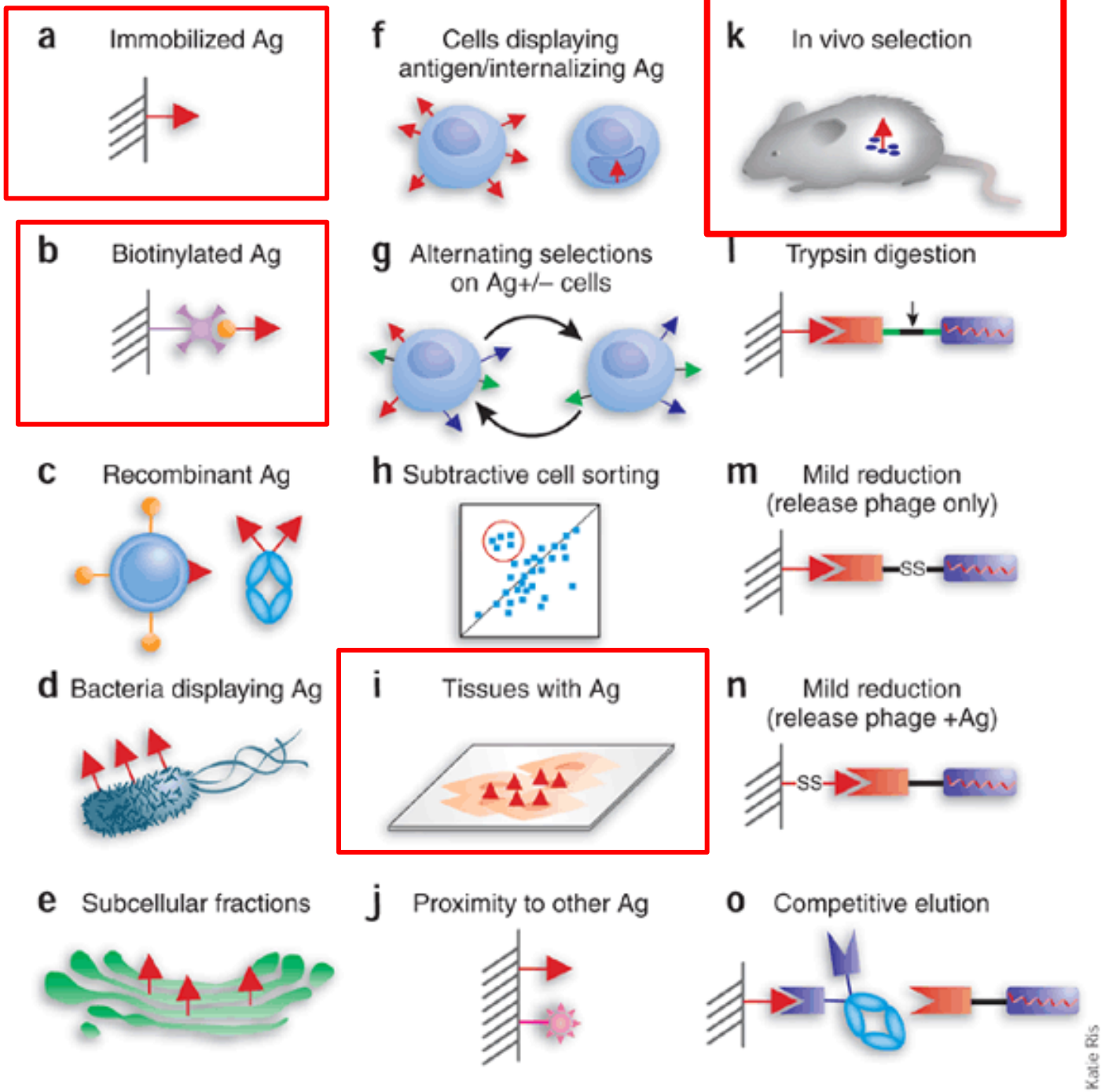
Reactive phage

Non-reactive phage



Dopo 3 cicli di selezione, recupero e caratterizzazione cloni reattivi




a Immobilized Ag
b Biotinylated Ag
c Recombinant Ag
d Bacteria displaying Ag
e Subcellular fractions
f Cells displaying antigen/internalizing Ag
g Alternating selections on Ag+/– cells
h Subtractive cell sorting
i Tissues with Ag
j Proximity to other Ag
k In vivo selection
l Trypsin digestion
m Mild reduction (release phage only)
SS
n Mild reduction (release phage +Ag)
SS
o Competitive elution
Katie Ris

ARTHRITIS & RHEUMATISM
Vol. 46, No. 8, August 2002, pp 2109–2120
DOI 10.1002/art.10464

Identification of Synovium-Specific Homing Peptides by In Vivo Phage Display Selection

Lewis Lee,[1] Christopher Buckley,[2] Mark C. Blades,[1] Gabriel Panayi,[1] Andrew J. T. George,[3] and Costantino Pitzalis[1]

Objective. **To identify homing peptides specific for human synovium that could be used as targeting devices for delivering therapeutic/diagnostic agents to human joints.**

Methods. **Human synovium and skin were transplanted into SCID mice. A disulfide-constrained 7–amino acid peptide phage display library was injected intravenously into the animals and synovial homing phage recovered from synovial grafts. Following 3–4 cycles of enrichment, DNA sequencing of homing phage clones allowed the identification of specific peptides that were synthesized by a-fluorenylmethyloxycarbonyl chemistry and used in competitive in vivo assays and immunohistochemistry analyses.**

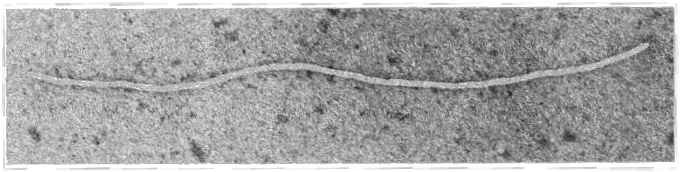
Results. **We isolated synovial homing phages displaying specific peptides that distinctively bound to synovial but not skin or mouse microvascular endothelium (MVE). They retained their tissue homing specificity in vivo, independently from the phage component, the original pathology of the transplanted tissue, and the degree of human/murine graft vascularization. One such peptide (CKSTHDRLC) maintained synovial homing specificity both when presented by the phage and as a free synthetic peptide. The synthetic peptide also competed with and inhibited in vivo the binding of the parent phage to the cognate synovial MVE ligand.**

Conclusion. **This is the first report describing peptides with homing properties specific for human synovial MVE. This was demonstrated using a novel approach targeting human tissues, transplanted into SCID mice, directly by in vivo phage display selection. The identification of such peptides opens the possibility of using these sequences to construct joint-specific drug delivery systems that may have considerable impact in the treatment of arthritic conditions.**

The microvascular endothelium (MVE) plays a major role in the pathogenesis of rheumatoid arthritis (RA), making it an important therapeutic target. RA is a condition characterized by a proliferative synovitis that is responsible for cartilage and bone damage leading to progressive joint destruction (1,2). Florid sprouting of new blood vessels (neoangiogenesis) is typically seen in the early phases of RA synovitis, suggesting that it is a critical element in this condition (3). In the established chronic phase of the disease, the MVE is also important, since it functions as a conduit for the continuous influx of inflammatory cells from the bloodstream into the

Phage antibodies vs. hybridomas

- Antibodies from any species
- In vitro
- Immune or naive source
- Billions antibodies screened
- Affinities $\leq 10^{-8}$
- Affinities can be improved $\leq 10^{-9}$
- Libraries difficult to make and use
- Conserved antigens possible
- High throughput possible
- Gene cloned with selection
- Unusual selection strategies



- Rodent antibodies only
- Requires animals
- Immune source needed
- Hundreds antibodies screened
- Affnities $\leq 10^{-10}$
- Cannot improve affinity
- Technology widely available and works well
- Conserved antigens difficult
- High throughput impossible
- Gene must be cloned separately
- Only immunisation

Antibodies from library: advantages 1

- **Antibodies against self or very conserved antigens possible** (e.g. thyroglobulin, TNFa, CEA, MUC1, CD4)
- **Impossible antibodies possible** (e.g. BIP)
- Antibodies with **fine discrimination** (e.g. estrogen, estradiol, targets differing by a single amino acid)
- Antibodies with **very broad specificities** (e.g. post-translational modifications independently of sequence context)
- **Unusual selection strategies** - Subtractive antibodies, cell surface antibodies
- **Recombinant clones: gene cloned with selection**
  - Availability of sequence means selected affinity reagents are never lost, but archivable forever
  - With improvement of gene synthesis, web based distribution of clone sequences can be contemplated in the future
- **Easily manipulated for downstream uses**
  - Enzyme fusions, multimerization etc.

Antibodies from library: advantages 2

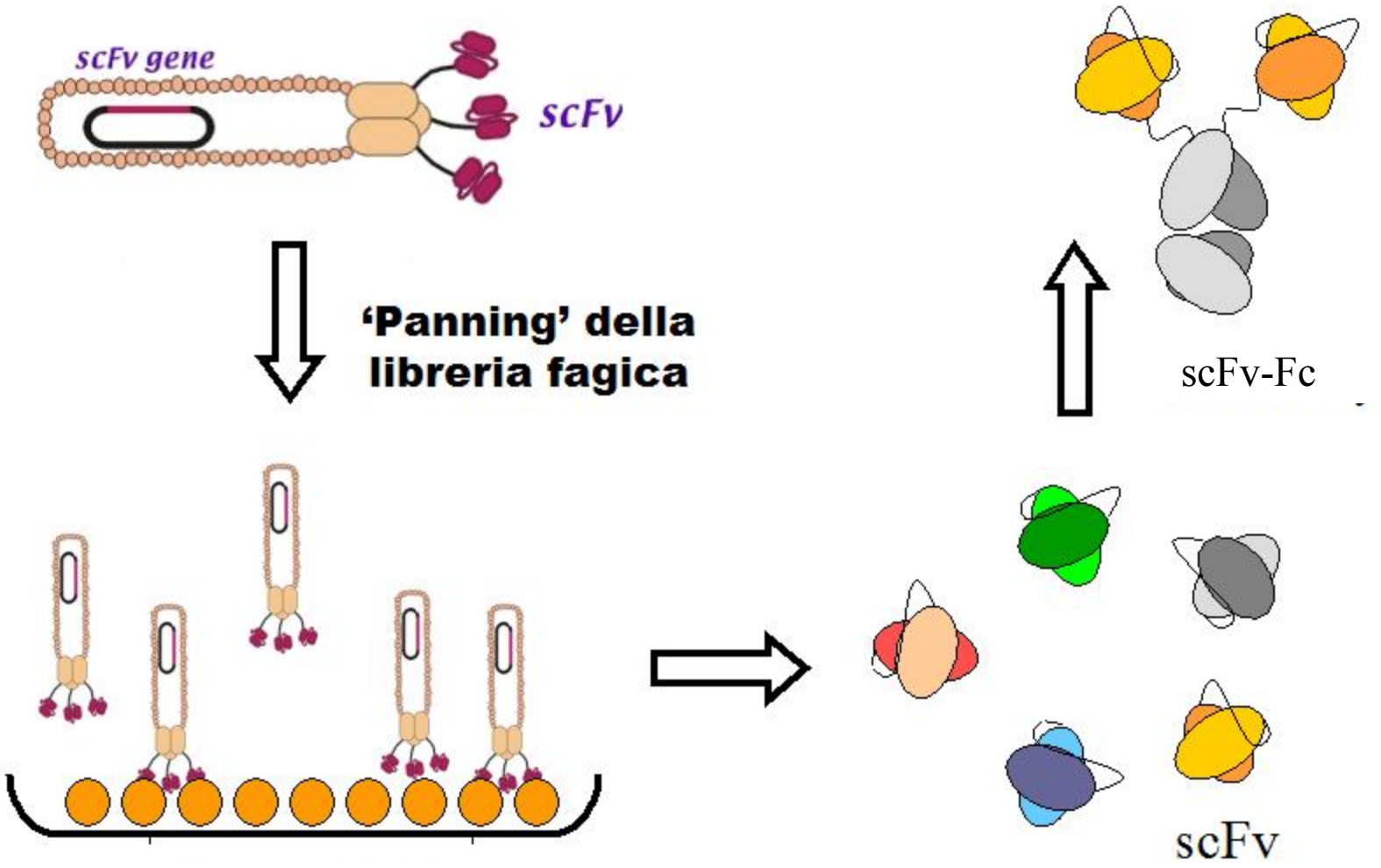
- **Isolate numerous antibodies per antigen in two weeks** (simple selections)
- **Process multiple antigens**: ≥10 manually, 96 well format feasible
- Antibodies isolated can be **high affinity**
- Affinity can be further improved
- Human antibodies can be isolated
- **Affinities 1nM-10μM**
  - Depends upon library quality and degree of effort
  - Can affinity mature to picomolar
- **Truly monoclonal**, make multimeric or polyclonal or oligoclonal as desired
  - Hybridomas often produce multiple different antibodies
  - Less than 33% of commercial antibodies recognize their targets (M. Uhlen - protein atlas)
- **Can be converted back to IgG of any species**

Antibodies from library: disadvantages

- Obtaining or making a library
- Classical monoclonal antibodies work well
- Technique can be difficult to use
- ScFv are produced in bacteria after selection
  - Yields 100μg-10mg/litre
  - scFvs tend to have low stability / storage capability
- Affinities 1nM-10μM
  - Depends upon library quality and degree of effort
- Antibodies are monomeric

Points to consider when making and using phage based antibody libraries

- Antibody form (Single chain Fv (scFv) or Fab)
- The source of V region diversity (natural or synthetic)
- For natural V genes, the tissue source of V genes
- How to assemble the V regions
- The display protein used (p3, p8 or p9)
- Phage or phagemid
- Selection strategy
- **Screening**

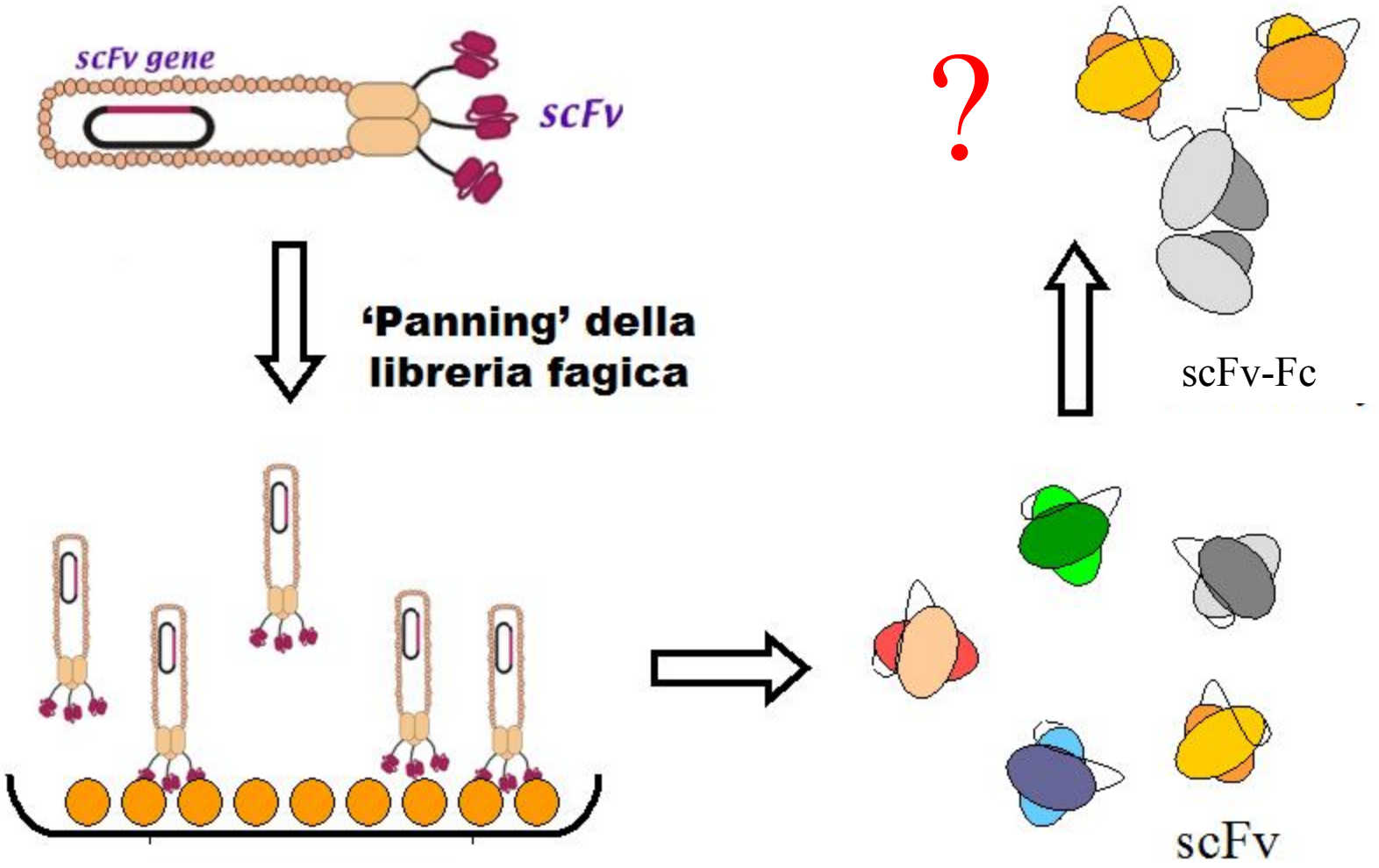

scFv gene
scFv
'Panning' della libreria fagica
scFv-Fc
scFv
?

Antibody screening: tests

- ELISA (Using supernatant/extract)
- BIACORE (affinity using purified molecules)

- Western blot (or immunoistochemistry, or….)

- Functional screening


scFv gene
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?
'Panning' della
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scFv

Antibody modifications

- Increase affinity
  - Chain shuffling
  - Point mutation

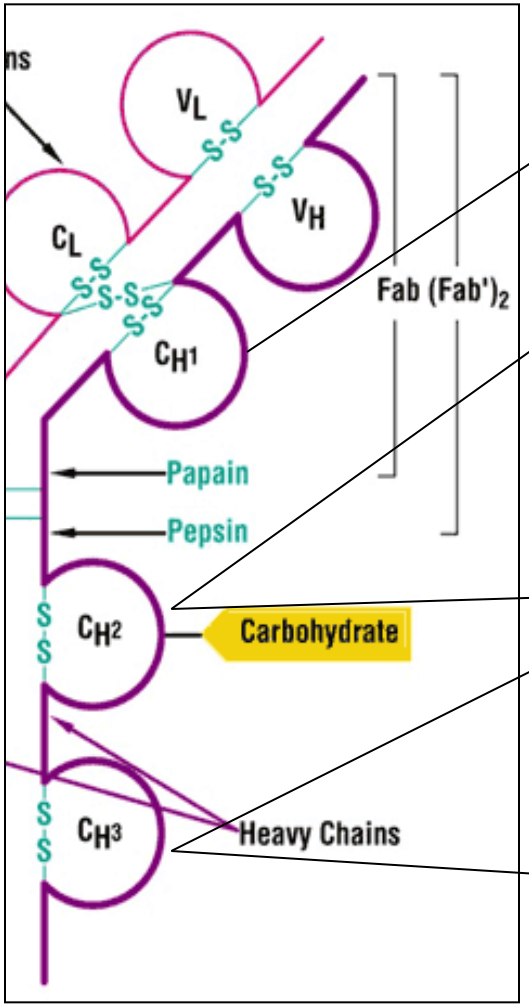
- Modification to Fc portion
  - Change Fc (IgG, IgM, IgA; mouse, rat, dog, human)

Antibody modifications

- Increase affinity
  - Chain shuffling
  - Point mutation

- Modification to Fc portion
  - Change Fc (IgG, IgM, IgA; mouse, rat, human)
  - Enhance CDC
  - Enhance ADCC

Functions of Fc domain



CH1 : - structural function

CH2 : - Complement activation
- ADCC

CH2 - phagocytosis
CH3 - clearance (kidneys)
- placental activity

CH3 : - MAST-cell binding

J Clin Invest. 2015 Oct 5. pii: 82695. doi: 10.1172/JCI82695. [Epub ahead of print]

Sialylation of IgG Fc domain impairs complement-dependent cytotoxicity.

Quast I, Keller CW, Maurer MA, Giddens JP, Tackenberg B, Wang LX, Münz C, Nimmerjahn F, Dalakas MC, Lünemann JD.

Abstract

IgG molecules exert both pro- and antiinflammatory effector functions based on the composition of the fragment crystallizable (Fc) domain glycan. Sialylated IgG Fc domains have antiinflammatory properties that are attributed to their ability to increase the activation threshold of innate effector cells to immune complexes by stimulating the upregulation of the inhibitory Fcγ receptor IIB (FcγRIIB). Here, we report that IgG Fc sialylation of human monoclonal IgG1 molecules impairs their efficacy to induce complement-mediated cytotoxicity (CDC). Fc sialylation of a CD20-targeting antibody had no impact on antibody-dependent cellular cytotoxicity and did not change the affinity of the antibody for activating Fcγ receptors. In contrast, the presence of sialic acid abrogated the increased binding of C1q to Fc-galactosylated IgG1 and resulted in decreased levels of C3b deposition on the cell surface. Similar to monoclonal antibodies, sialic acid inhibited the increased C1q binding to galactosylated Fc fragments in human polyclonal IgG. In sera derived from patients with chronic inflammatory demyelinating polyneuropathy, an autoimmune disease of the peripheral nervous system in which humoral immune responses mediate tissue damage, induction of IgG Fc sialylation was associated with clinical disease remission. Thus, impairment of CDC represents an FcγR-independent mechanism by which Fc-sialylated glycovariants might limit proinflammatory IgG effector functions.

PMID: 26436649 [PubMed - as supplied by publisher]

Choosing your production system

Antibody purification

- Using Fc portion
- Using specific Tag
- Using the antigen

Antibody modifications

- Increase affinity
  - Chain shuffling
  - Point mutation

- Modification to Fc portion
  - Change Fc (IgG, IgM, IgA; mouse, rat, human)
  - Enhance CDC
  - Enhance ADCC

- Production of bispecific antibody

Bispecific antibodies