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IMMUNO- GENETICS

2009/4

Study materials at : <http://www.zoologie.upol.cz/zam.htm>

LECTURE OVERVIEW

Basic immunogenetic terminology

- specificity and polymorphism
- immunoglobuline gene superfamily
- immunogenetics

Imunoglobulins, BCR, TCR

- structure, polymorphisms
- B-cell receptor development
- T-cell receptor development
- gene rearrangement, allelic exclusion

MHC glykoproteins

- structure, function
- gene coding MHC molecules (HLA)

TOLERANCE

SELF [own], regular
cells and molecules

IMMUNE SURVALIANCE

OWN dead, damaged or
abnormal cell and molecules



Foreign [non-self] substance

Protection against potential pathogens [disease-causing invaders]

DEFENSE

SPECIFICITY of immune system

The key feature
of the adaptive immune system
is

SPECIFICITY

The immune system is able distinguish
between antigens or small parts
of macromolecular antigens

SPECIFICITY of immune system

Fine specificity is attributed to highly variable

LYMPHOCYTE RECEPTORS

and

IMMUNOGLOBULINS

Those may bind to one molecule
but not to another
with only minor structural differences
from the first

POLYMORPHISM of immune system

Unique specificity of antigen receptors
and
the ability to react selectively
against a very broad range of foreign antigens
is the result of extensive

POLYMORPHISM

(Greek: *poly* =many, *morphe* =shape, structure)

Polymorphism means:

Existence of two or more alternative forms
or variants of expressed proteins in a population

GENETIC POLYMORPHISM

A broad repertoire of immune molecules is the result of:

Genetic polymorphism

POLYMORPHIC GENES



Alternative forms or variants are present in different members of the population [common variants are called alleles]

NONPOLYMORPHIC GENES



Genes represented by only one normal nucleic acid sequence in all members of a species

IMMUNOGLOBULIN SUPERFAMILY

Polymorphic immune molecules

belong to large family of proteins that contain a globular structure motif - Ig domain originally described in antibodies.

This group of proteins is called:

IMMUNOGLOBULIN SUPERFAMILY

IMMUNOGLOBULIN domain

Immunoglobulin domain (Ig domain):

- ❑ A three dimensional globular structural motif found in many proteins in the immune system
- ❑ About 110 aminoacid residues in length
- ❑ The principal elements of domain are two opposed β plated sheets stabilized with disulfide bonds (β barrel)

MEMBERS of immunoglobulin superfamily

There are about 40 members of immunoglobulin superfamily

- ❑ **Recognition and regulation molecules:**
Ig, BCR, TCR, MHC molecules,
CD2, CD3, CD4, CD8 molecules,
Fc receptors
- ❑ **Adhesion molecules :**
ICAM-1, ICAM-2, VCAM-1, PECAM-1
- ❑ **Receptors for PDGFR (= platelet growth factor)**

Immunoglobulin GENE superfamily

Genes that encode on cell surface molecules are part of

Immunoglobulin GENE SUPERFAMILY

- Appear to be evolutionary related genes
- Members of a family share a certain degree of sequence homology
- Are likely derived from a common precursor gene

IMMUNOGENETICS - discipline

The study of the GENETIC ASPECTS of the immune Response is called:

Immunogenetics

It focus on:

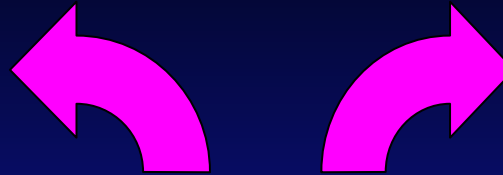
- Immune response genes [Ig gene superfamily]
- HLA antigens and their association with disease
- Generation of antibody diversity

Ig

IMMUNOGLOBULINS
[antibodies]

MHC

membrane
glykoproteins



The most

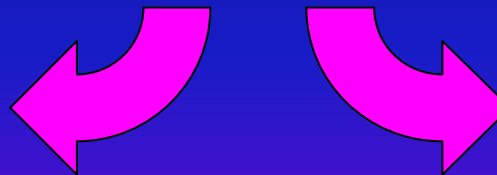
POLYMORPHIC MOLECULES

of the human immune system

(members of the immunoglobulin superfamily)

BCR

Membrane
receptor of
B lymphocyte



TCR

Membrane
Receptor of
T lymphocyte

Highly polymorphic immune molecules:

Ig

IMMUNOGLOBULINS
[antibodies]

BCR

Membrane receptor
of B lymphocyte

TCR

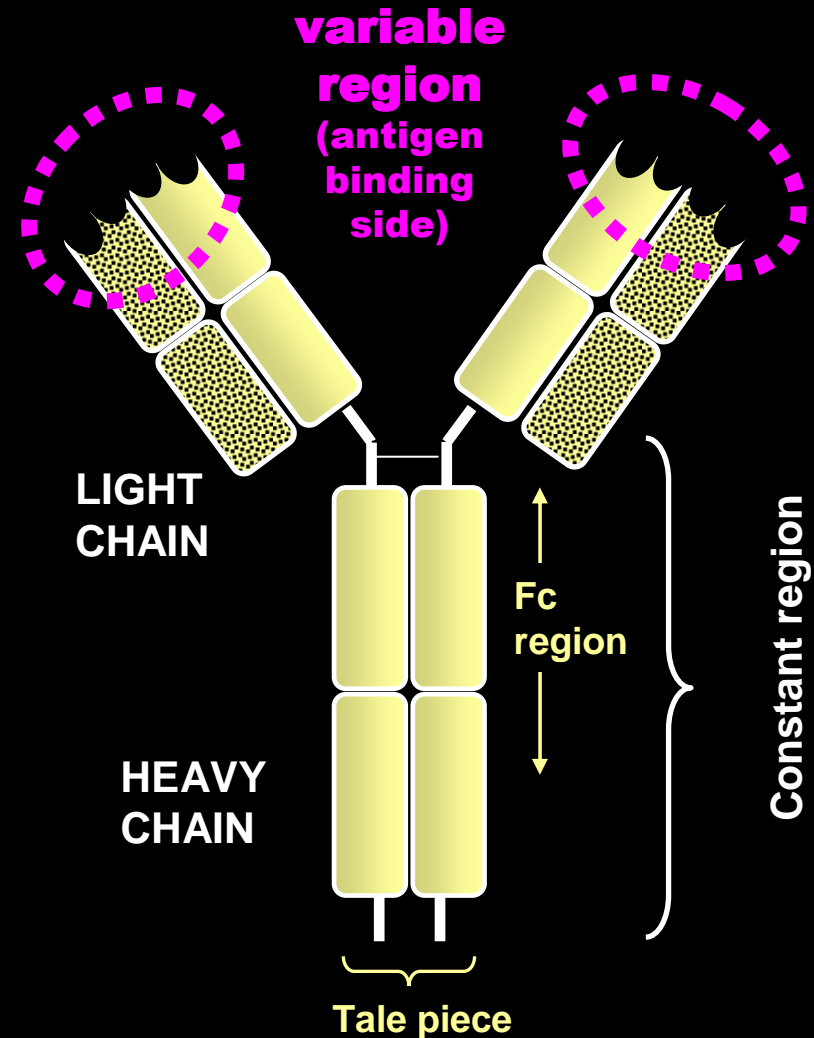
Membrane receptor
of T lymphocyte

A unique process during development and maturation
allows for the generation of
enormous diversity

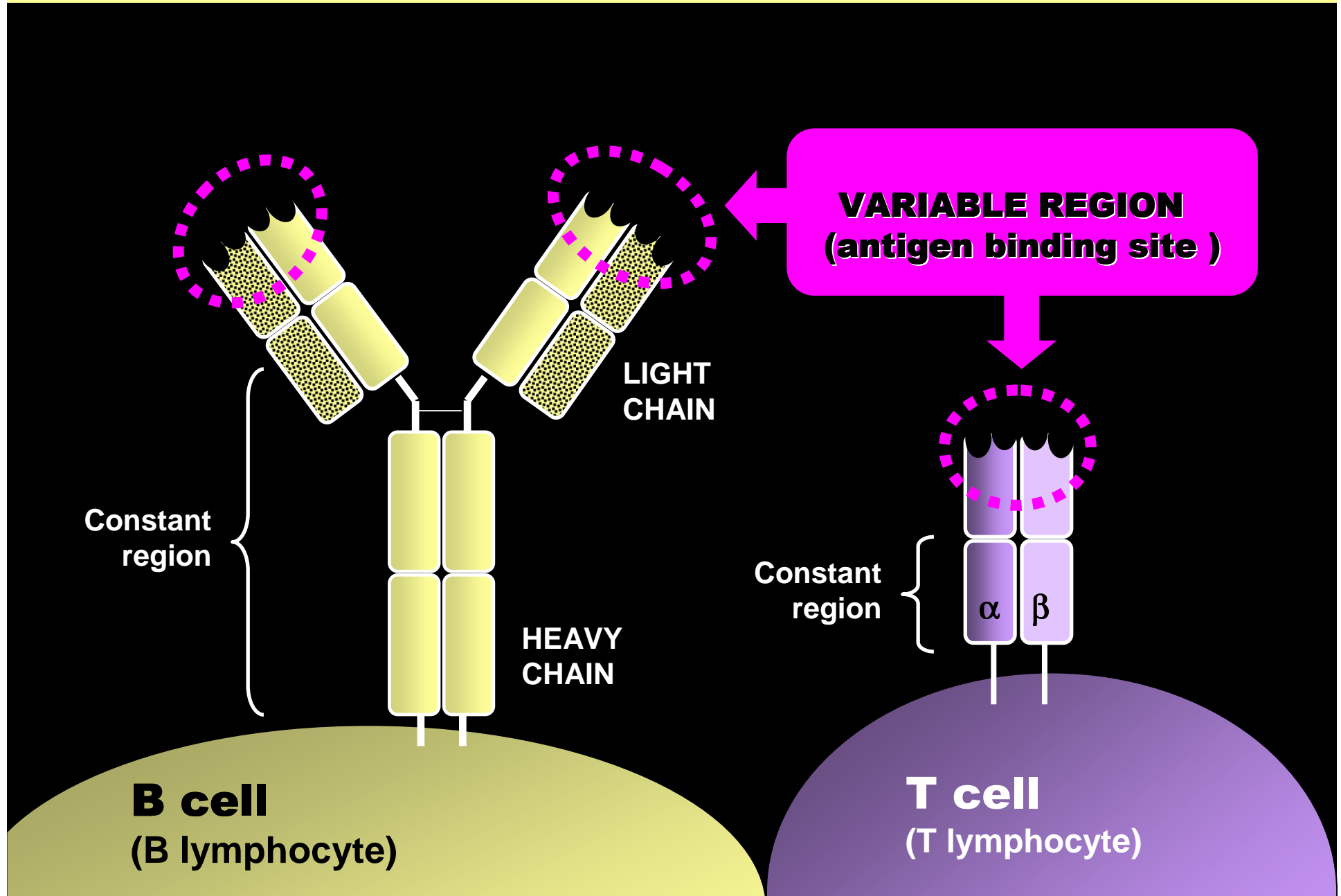
ANTIBODY

= immunoglobulins = gama-globulins

- ❑ Glycoproteins found in serum
- ❑ Y-shape molecule made of two identical heavy chains and two identical light chains
- ❑ Constant region (C-terminal)
- ❑ Variable regions (N-terminal) form antigen binding sites which specifically bind particular antigens



Membrane receptors of lymphocyte



Potential to create a huge number of
different antibodies and membrane receptors
protect a person against

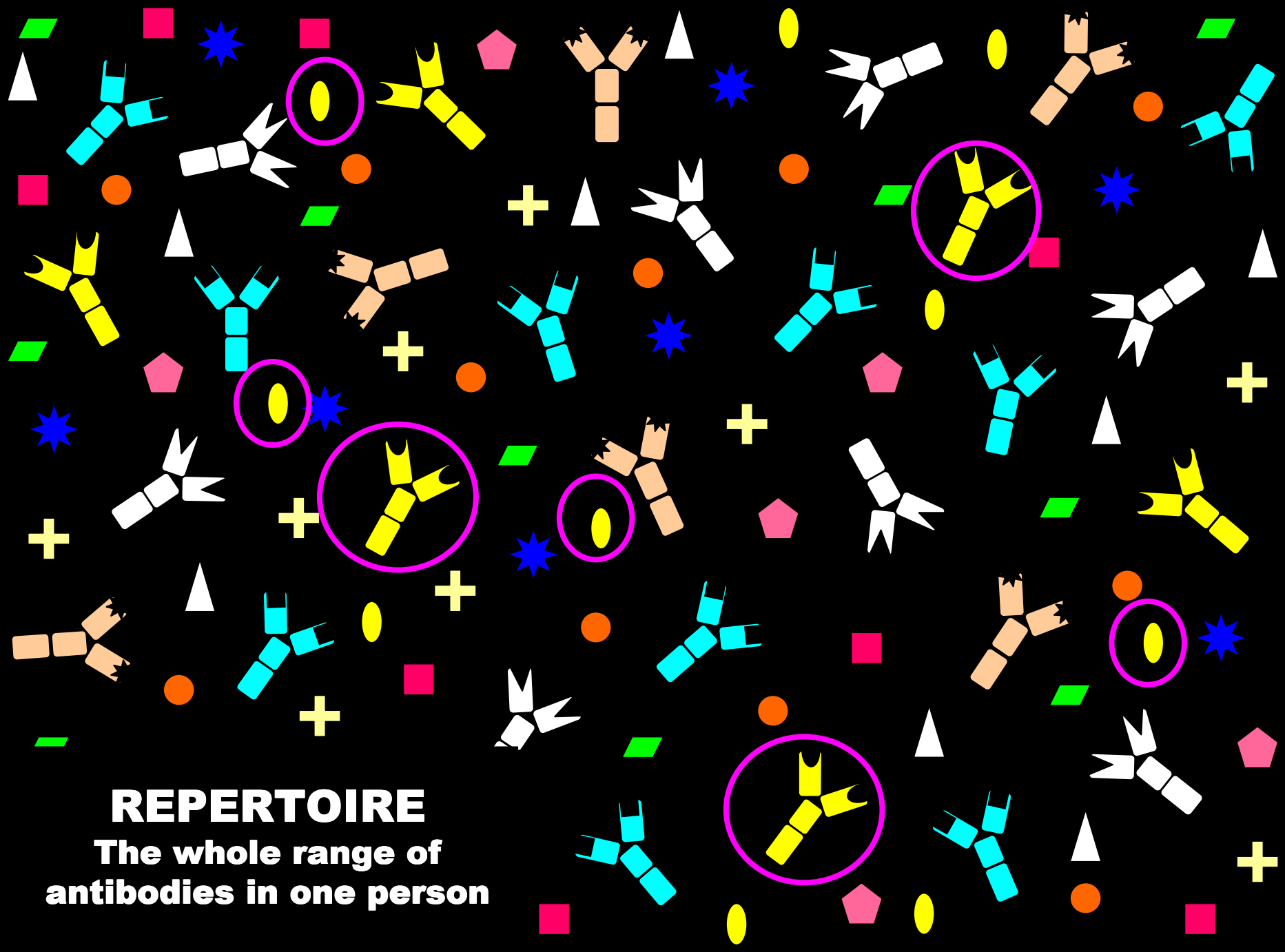
the large array of

infection organisms

toxic agents

autologous malignant cells

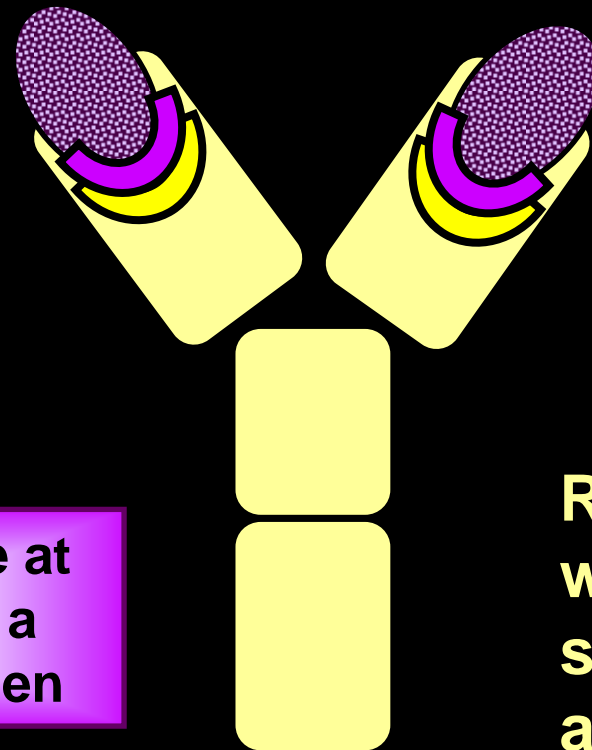
to which a person may be exposed



REPertoire
The whole range of
antibodies in one person

COMPLEMENTARITY: variable region - epitope

Sequence at the variable region of a receptor is compatible to....



Antigen which specifically binds to a particular receptor

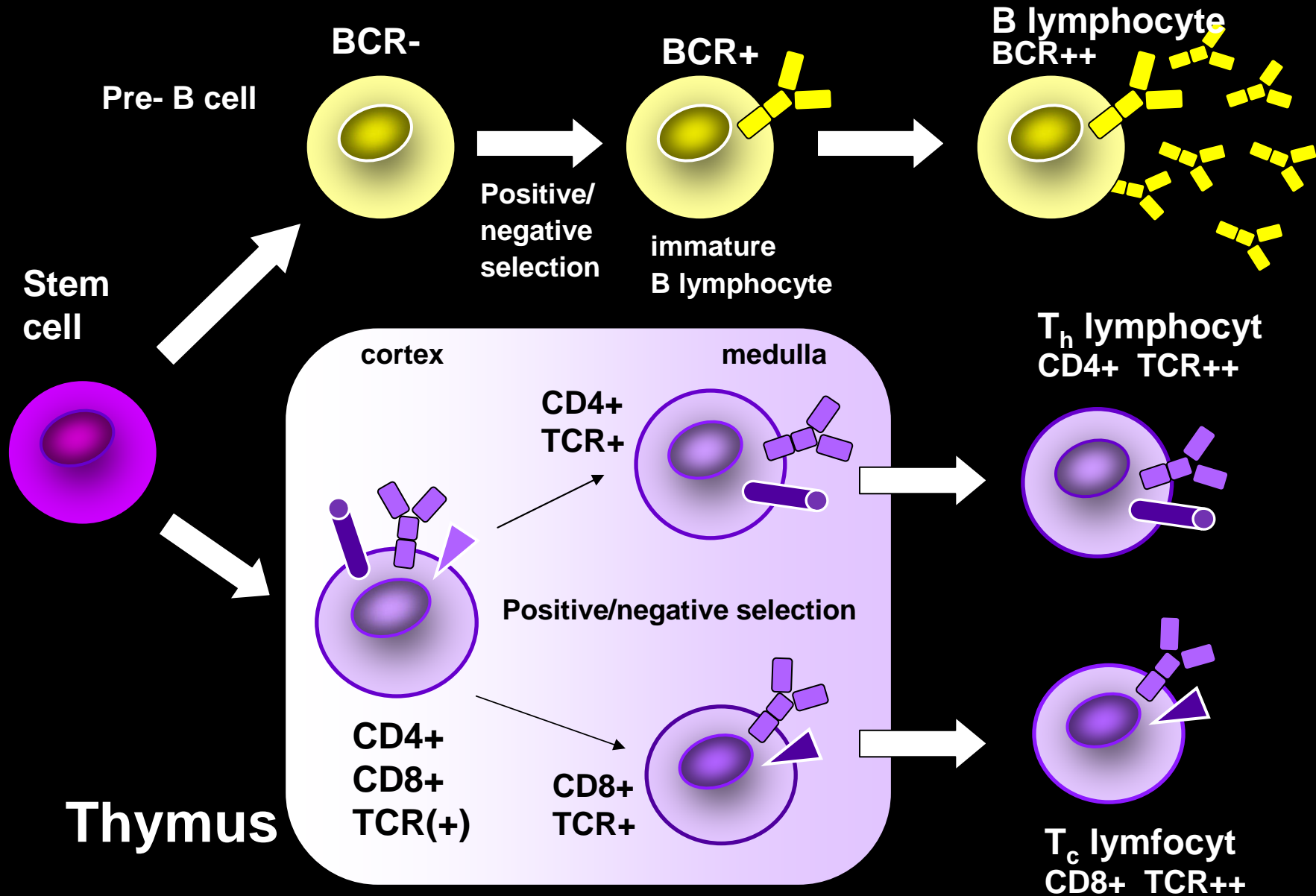
... the sequence at the epitope of a particular antigen

RECEPTOR (antibody) with variable region specific to a particular antigen

OVERVIEW OF LYMPHOCYTE DEVELOPMENT

Central lymphoid organs: bone marrow, thymus (liver in fetus)

Peripheral lymphatic organs



Ig, BCR and TCR are encoded in the germline
by a relatively small number of genes

The generation of enormous diversity allows
a unique process during development and maturation :

**Gene segment
rearrangement**

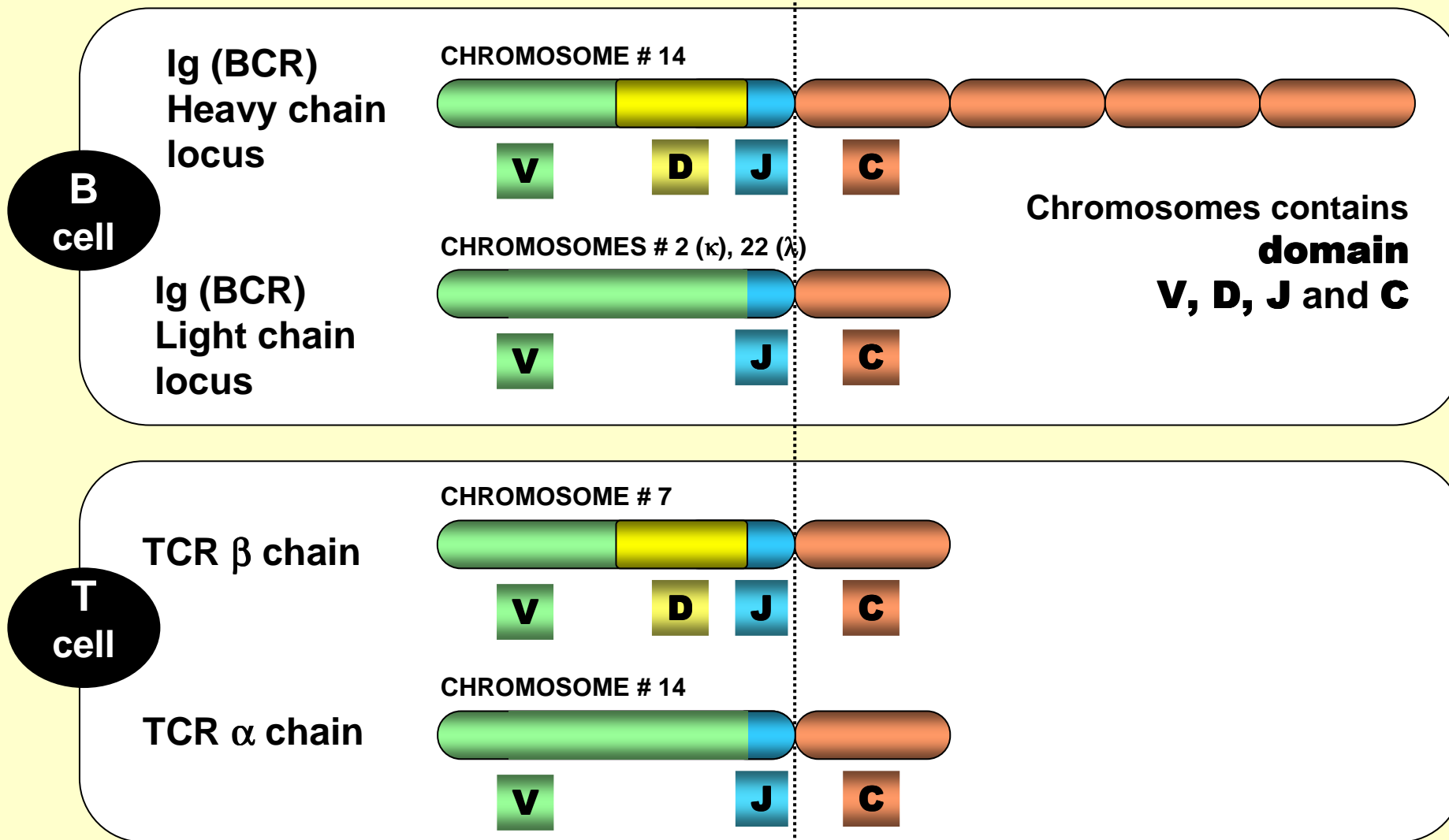
and

somatic mutation

Genes encoding Ig, BCR and TCR are on chromosomes 2, 14, 22

GENES encoding Ig, BCR and TCR: germline organization

Chromosomes
2, 14 and 22



Gene segment REARRANGEMENT

Each domain has many segments

„variability“ > 100

„diversity“ ~ 50

„joining“ 9

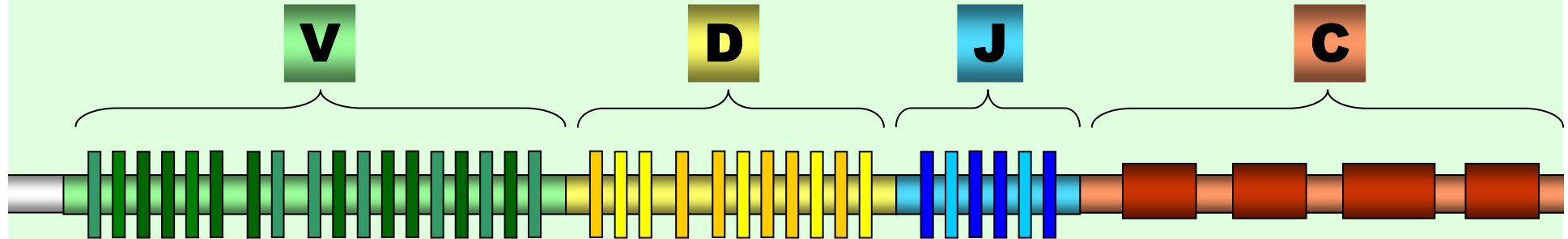
Gene encoding constant region

V

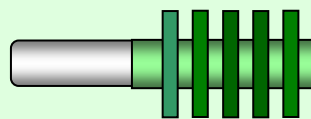
D

J

C

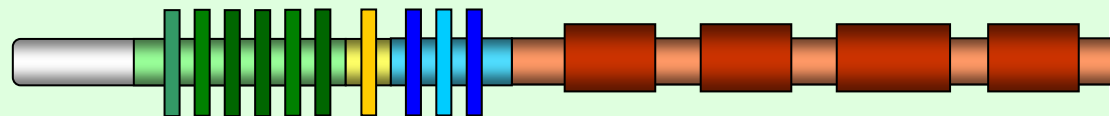


D – J
rearrangement

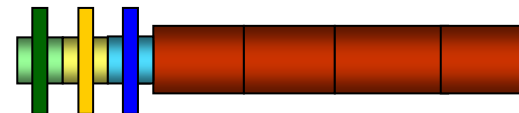


V – D rearrangement

splicing



V – D – J rearranged DNA, which serves as a template for transcription and translation



HEAVY CHAIN

LIGHT CHAIN

Germline DNA

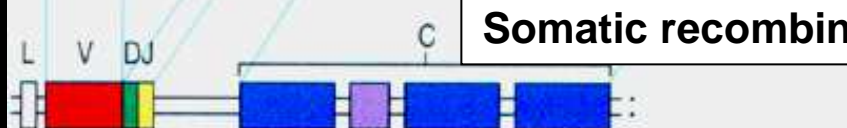
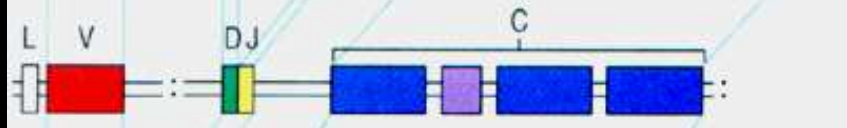
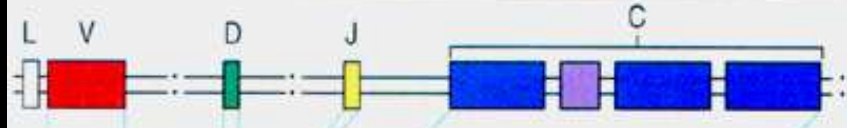
D – J joined rearranged DNA

V – D joined rearranged DNA

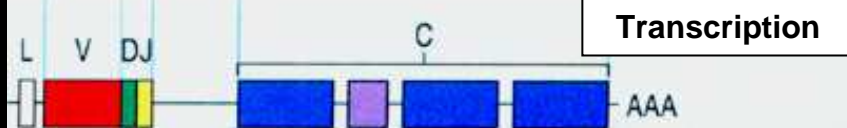
Primary transcript RNA

mRNA

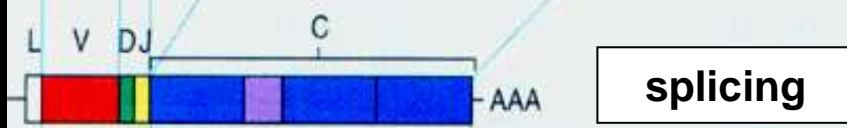
Polypeptide chain



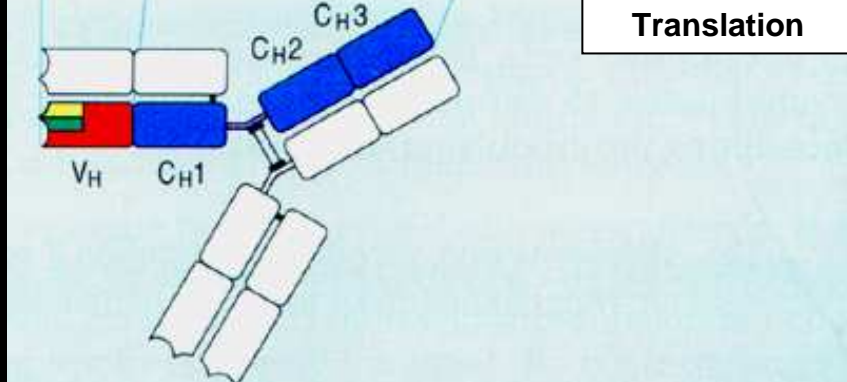
Somatic recombination



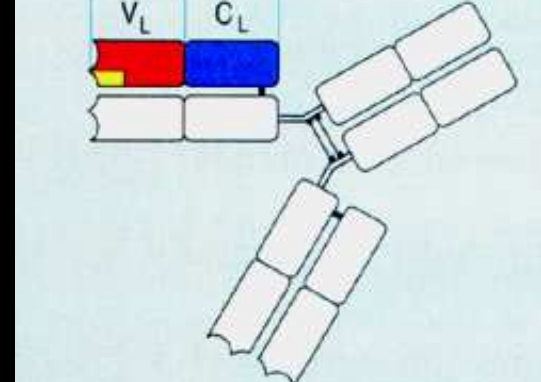
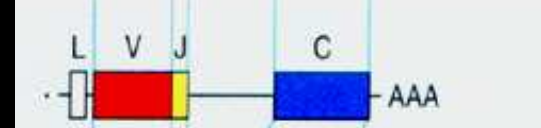
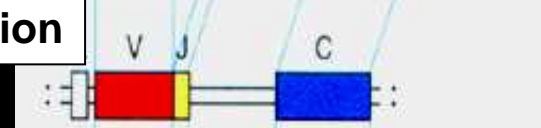
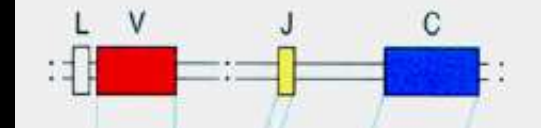
Transcription



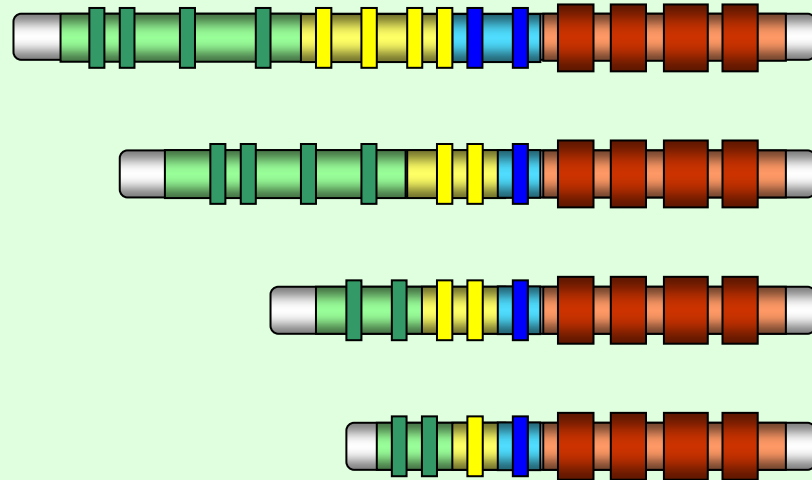
splicing



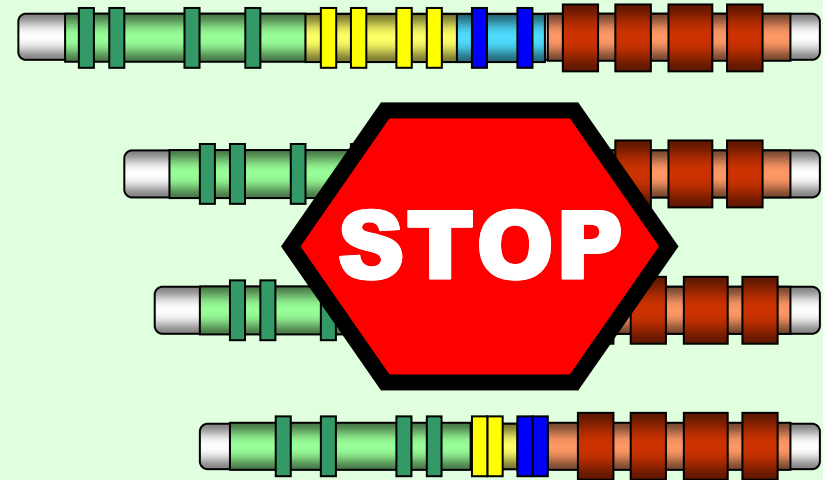
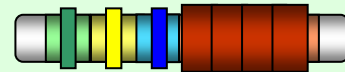
Translation



Gene segment rearrangement happens
on both homologue chromosomes at the same time



correct V-D-J-C
sequence



Rearrangement on the pair hom.
chromosome is terminated

Allelic exclusion

Contribution of different mechanisms to the generation of diversity in Ig and TCR genes

Element	IMMUNOGLOBULINS		TCR	
	H	$\kappa+\lambda$	β	α
Variable segments (V)	65	70	52	~70
Diversity segments (D)	27	0	2	0
D segments read in 3 frames	rarely	—	often	—
Joining segments (J)	6	5(κ) 4(λ)	13	61
Joints with N- and P-nucleotides	2	50% of joints	2	1
Number of V gene pairs	3.4 x 10 ⁶		5.8 x 10 ⁶	
Junctional diversity	~3 x 10 ⁷		~2 x 10 ¹¹	
TOTAL DIVERSITY	~ 10¹⁴		~ 10¹⁸	

MHC

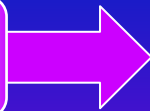
=

HLA

Major **H**istocompatibility **C**omplex

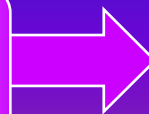
- Cluster of genes located on the short arm of chromosome 6
- Highly polymorphic gene encoding membrane MHC glycoproteins
- MHC in humans is called HLA complex (= Human Leukocyte Antigens)

WHERE?



MHC [HLA] molecules are found in all nuclear cells in humans

2 categories of MHC gene products in humans



Class I MHC glykoproteins
Class II MHC glykoproteins

Primary immunology function of

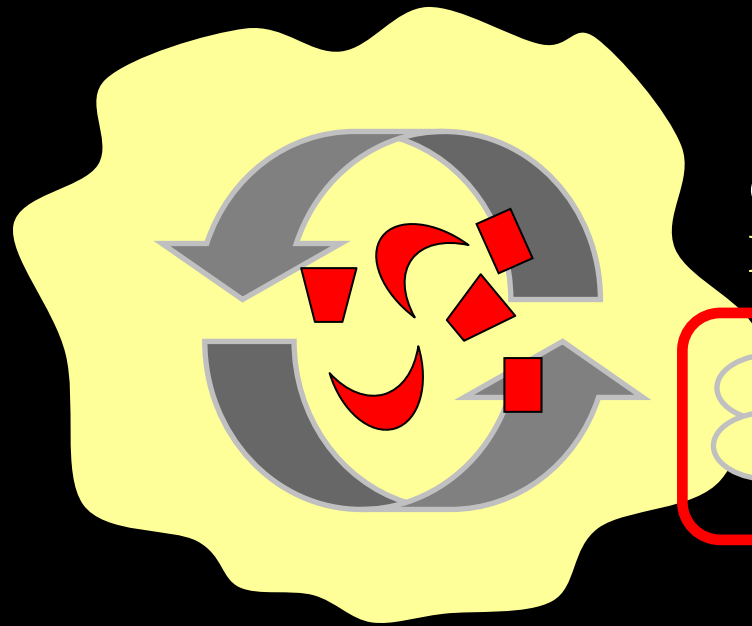
MHC molecules:

Sampling intracellular proteins

- ❑ **binding** to a peptide fragment derived from antigenic protein
- ❑ **presenting** a peptide fragment on the cell surface for recognition by the T cell receptor

Presentation of protein antigen to T cell

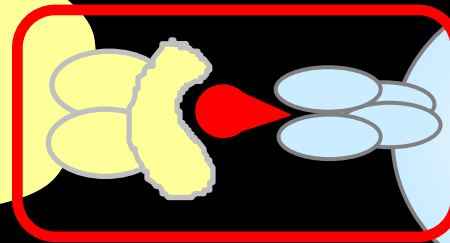
APC (Antigen presenting cell)



mediate
immune
response

Cytokine release

Complex
MHC-antigen



T cell

T cell

T cell

T cell

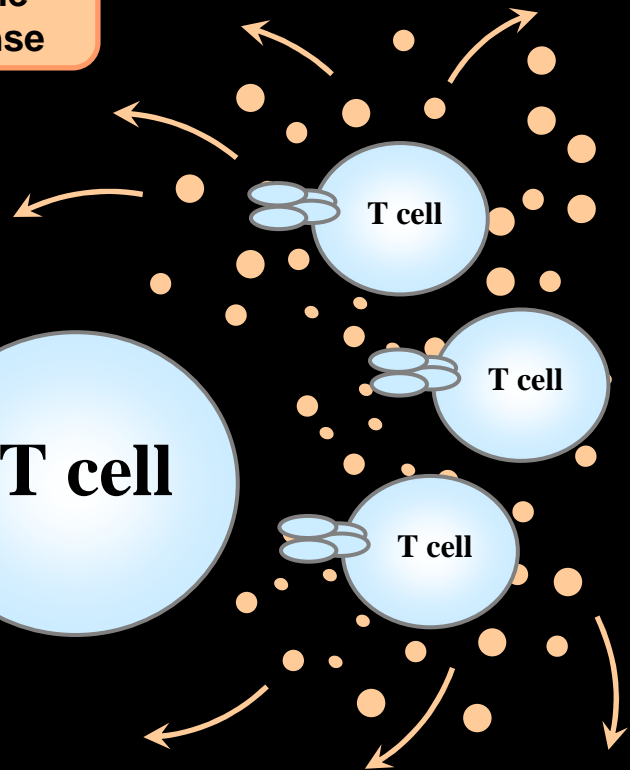
ANTIGEN

antigen
processing

antigen
presentation

T cell
stimulation

T cell
proliferation



T lymphocyte

T cell contact residue of peptide

T cell receptor

Polymorphic residue of MHC

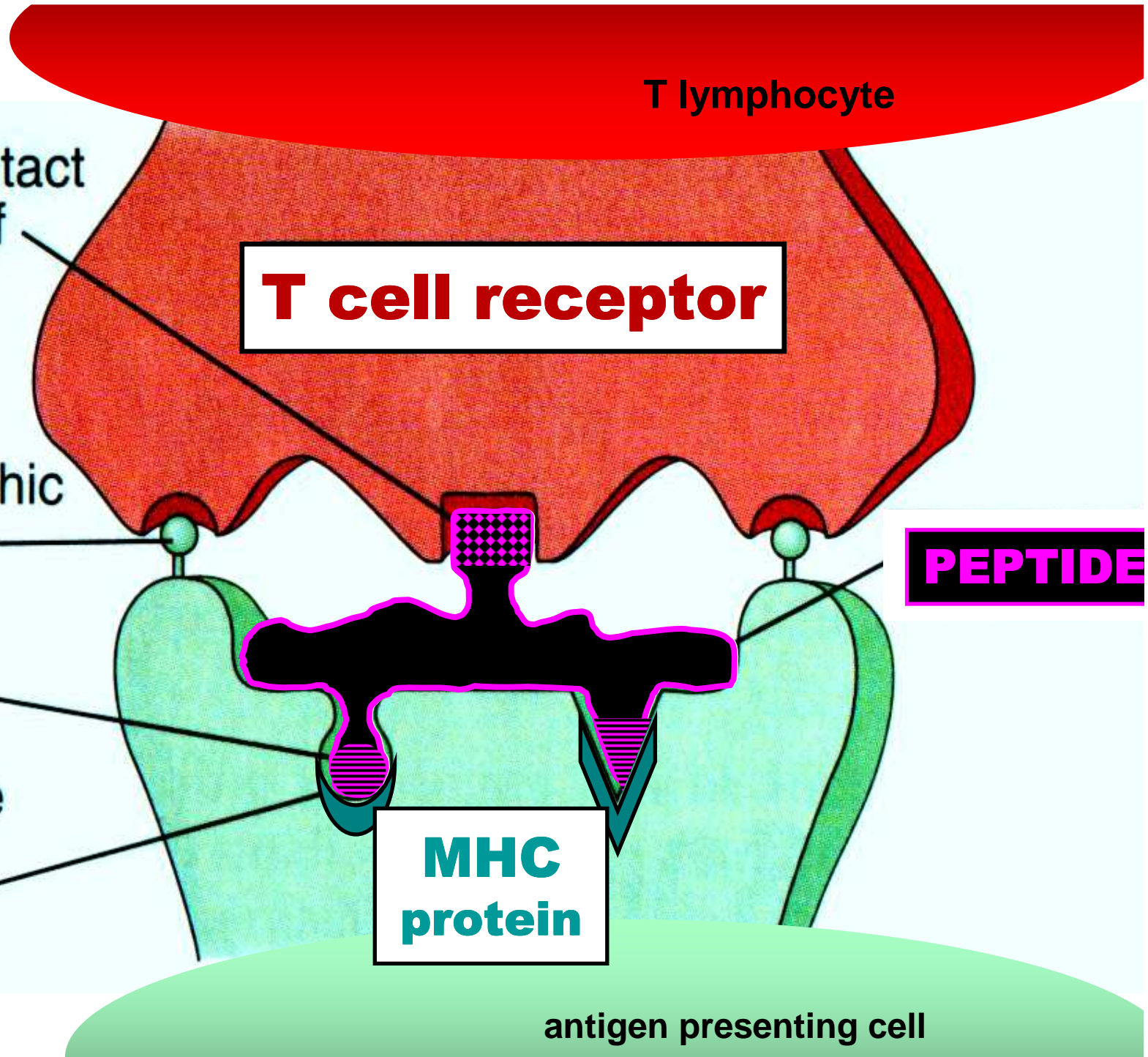
PEPTIDE

Anchor residue of peptide

"Pocket" of MHC

MHC protein

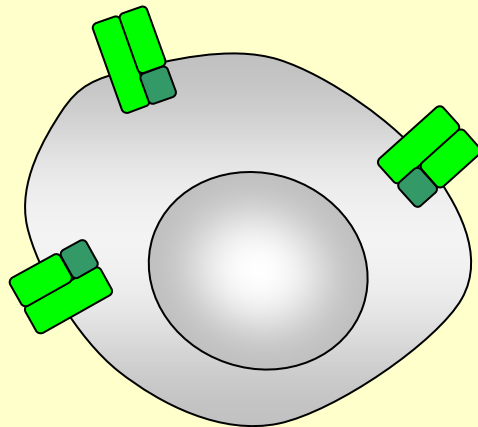
antigen presenting cell



Location of MHC molecules

MHC I

Class I glycoproteins

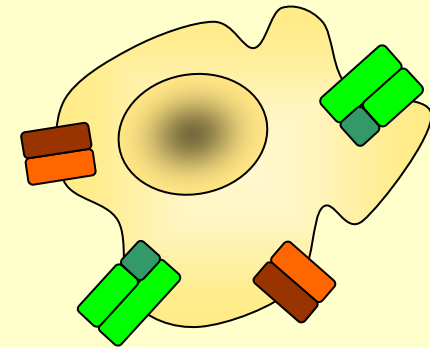
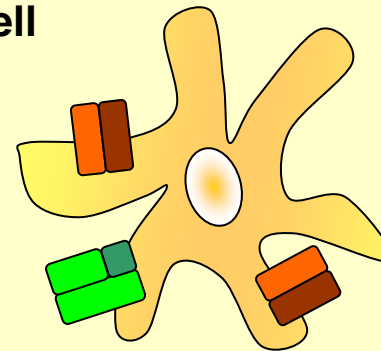


Expressed on the surface
of all nucleated cells

MHC II

Class II glycoproteins

Dendritic
cell



macrophages

**ONLY at antigen presenting cells -
APC(phagocytes, dendritic cells)**

Functional differences

GLYCOPROTEINS

MHC class I

Bind peptides derived from
cytosolic proteins

Bind peptides endogenously
synthesized by cells
[pathological or viral protein]

Are recognized by
cytotoxic T_c lymphocytes,
[CD8+ T cell]

GLYCOPROTEINS

MHC class II

Bind peptides derived from
phagosome or endosome

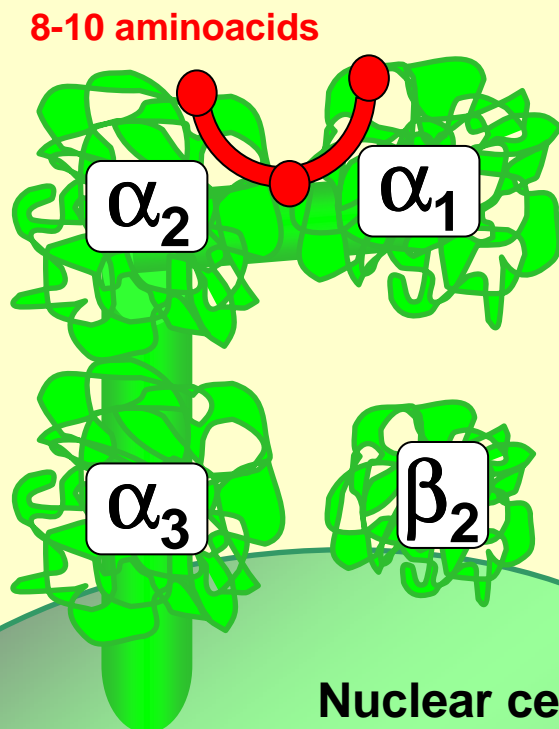
Bind exogenous peptides
derived from phagocytosed proteins
[mostly bacterial proteins]

Are recognized by
helper T_h lymphocytes
[CD4+ T cell]

Structure of MHC molecules

Class I MHC

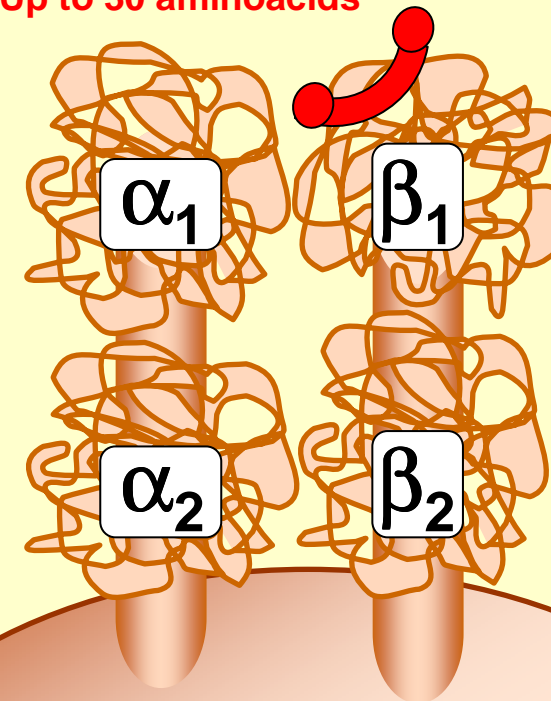
Polymorphic α chain
contains 3 domains (α_1 α_2 α_3)
Nonpolymorphic β_2 microglobulin



Class II MHC

Polymorphic α chain (domains α_1 α_2)
Polymorphic β chain (domains β_1 β_2)

Up to 30 aminoacids



Peptide
binding
cleft

Nuclear cell

Antigen presenting cell

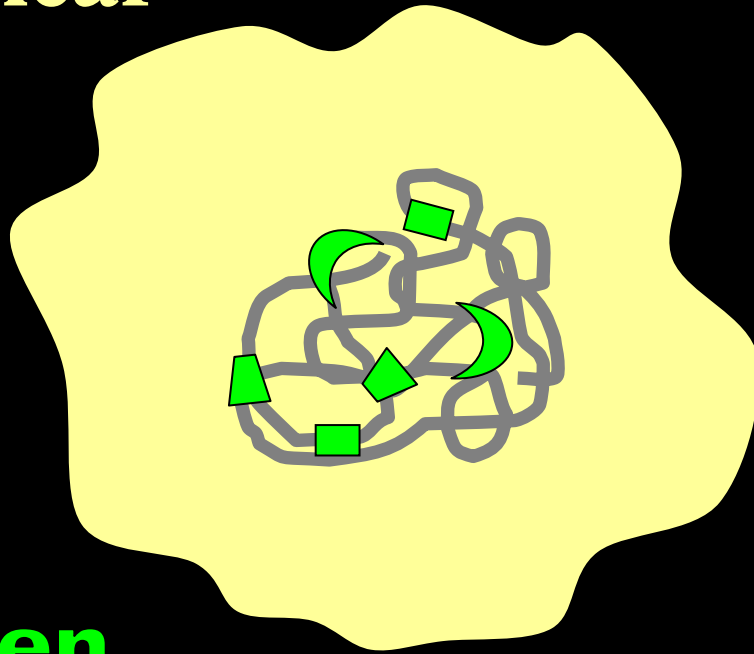
MHC

class I molecules

- **Handle intrinsic peptide antigens**
- **Present antigens to cytotoxic T cells**

1. Endogenous antigen

Nuclear
cell



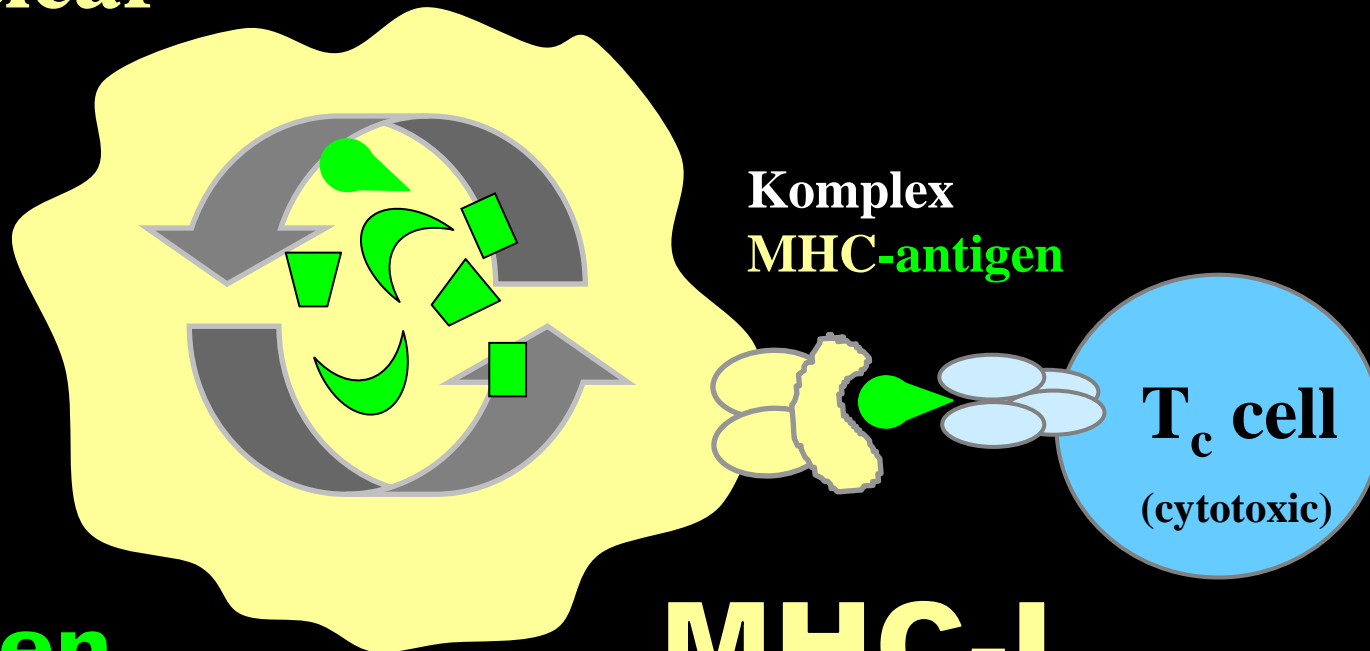
antigen

endogenously synthesized

- viral proteins
- mutated proteins (tumor cells)

1. Endogenous antigen

Nuclear
cell



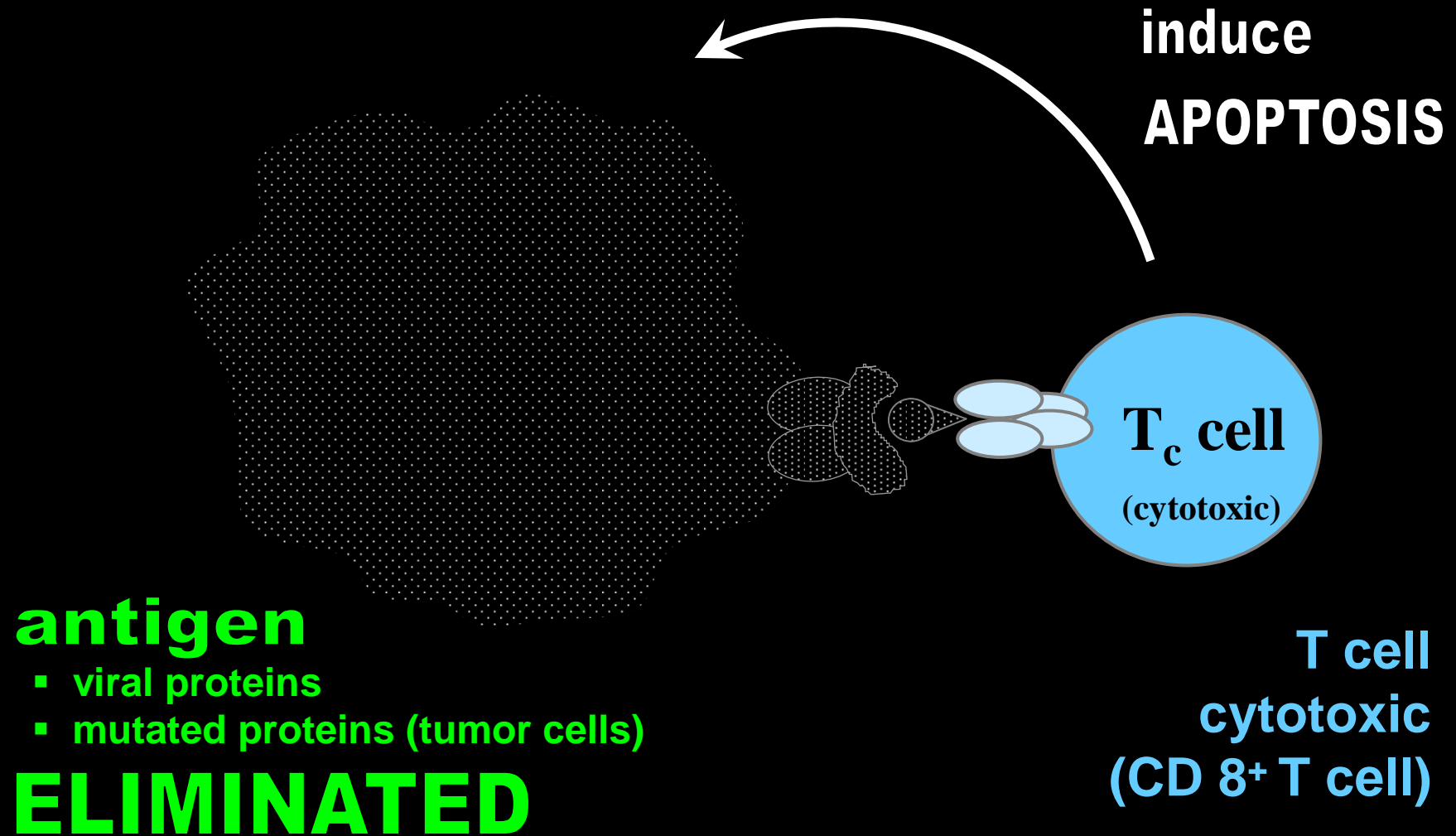
antigen
endogenously synthesized

- viral proteins
- mutated proteins (tumor cells)

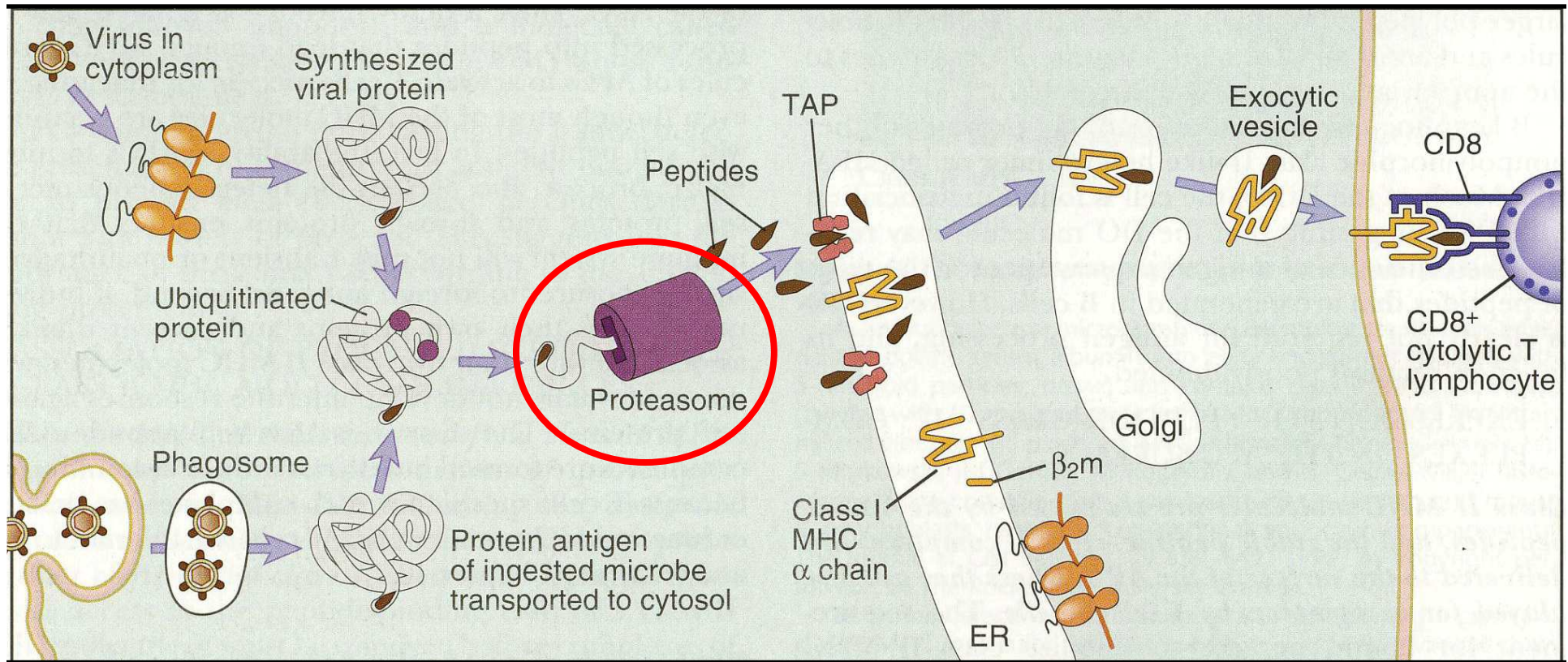
MHC-I

T cell
cytotoxic
(CD 8⁺ T cell)

1. Endogenous antigen



The class I MHC pathway of antigen presentation



Production of proteins

In cytosol:

1. Viral proteins
2. Protein antigen of digested microbe transported to cytosol
3. Neoplastic protein

Proteolytic degradation of proteins by proteasome

(a large proteolytic protease) in cytosol

Transport of peptides from cytosol to ER

(by TAP-transporter associated with antigen processing)

Assembly of peptide-class I complex (in ER)

Peptide class I complex is transported

through the Golgi apparatus to the cell surface

And presented to the CD8+ T cells

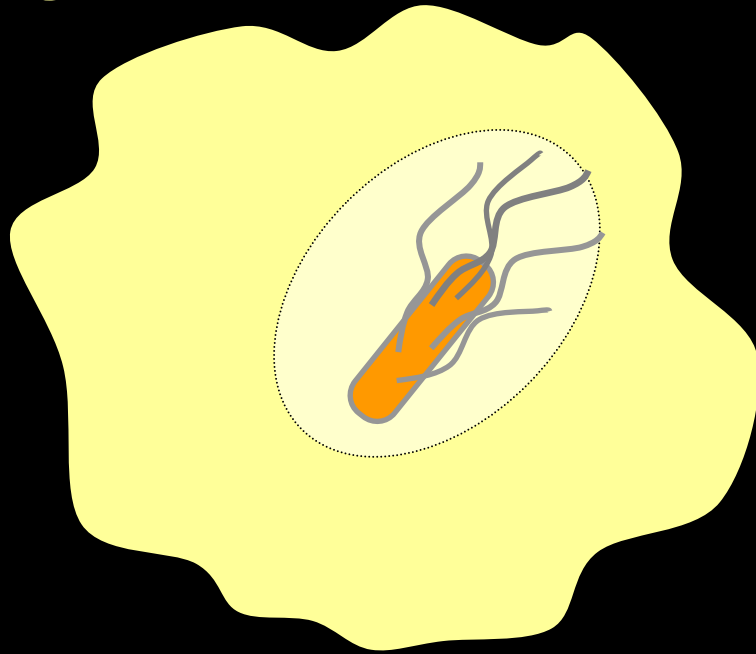
MHC

class II molecules

- **Handle extrinsic peptide antigens**
- **Present antigens to helper T cells**

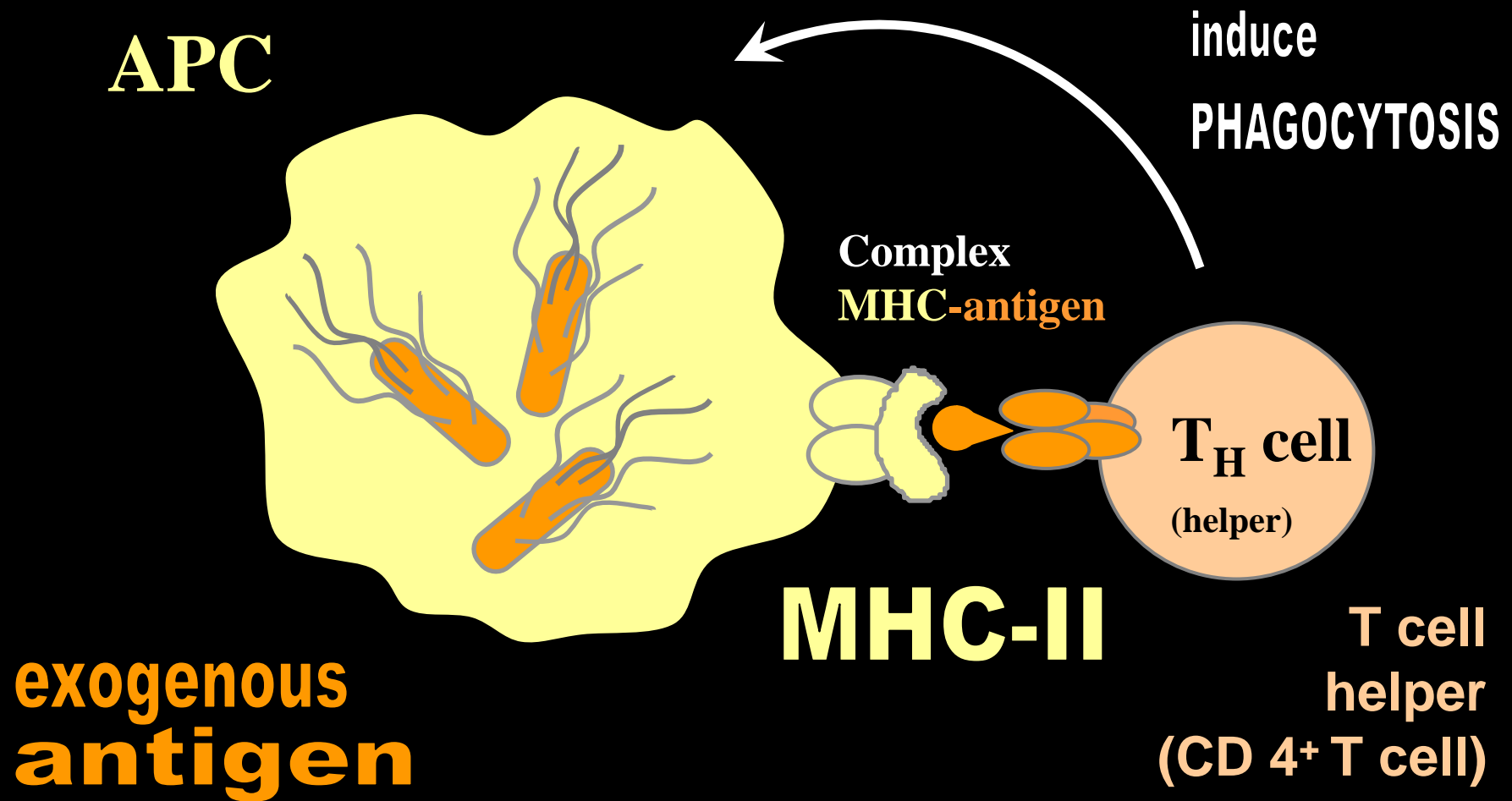
2. Exogenous antigen

APC

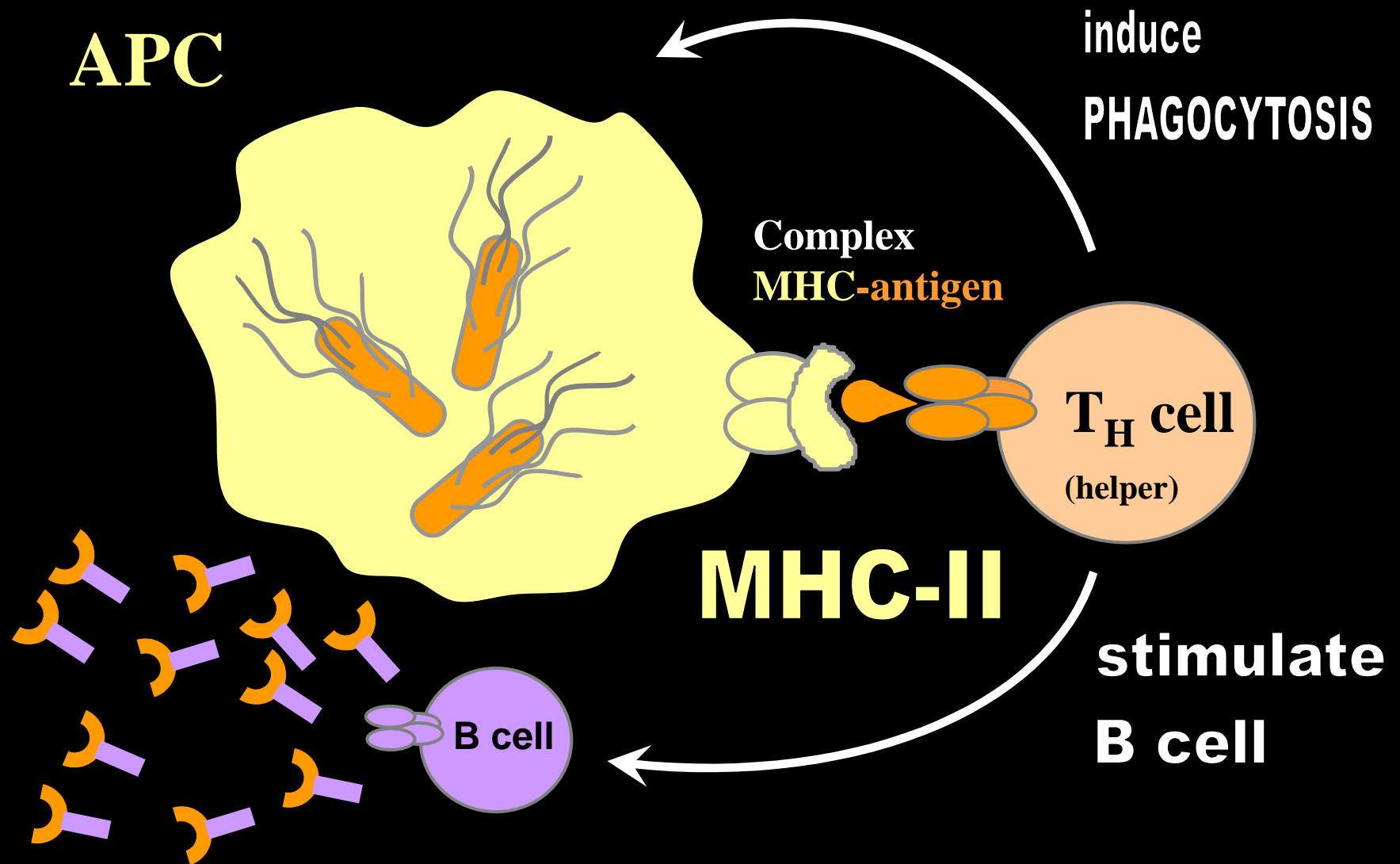


exogenous
antigen

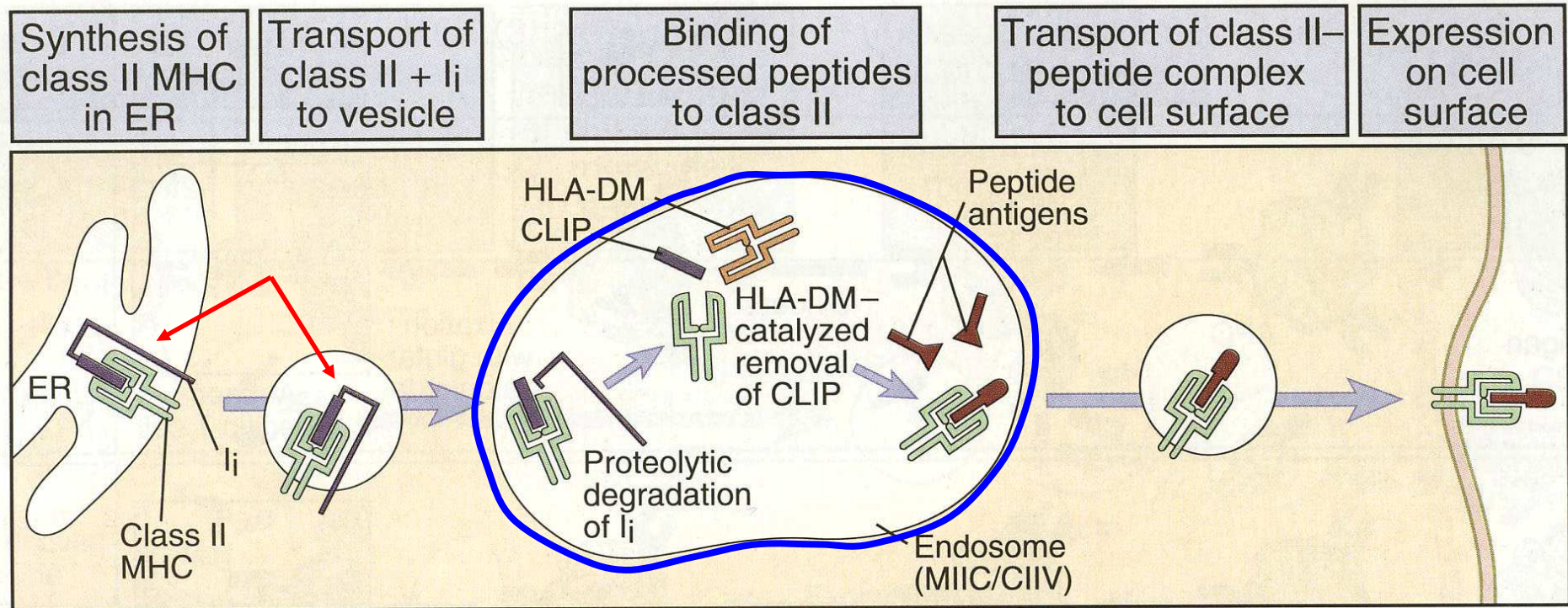
2. Exogenous antigen



2. Exogenous antigen



Several steps of antigen presentation to CD 4+ T cells



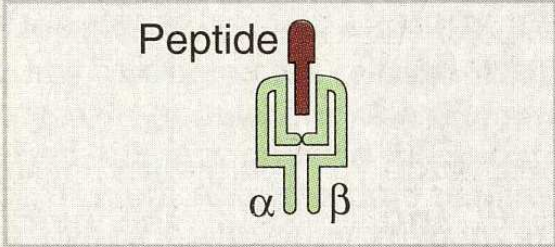
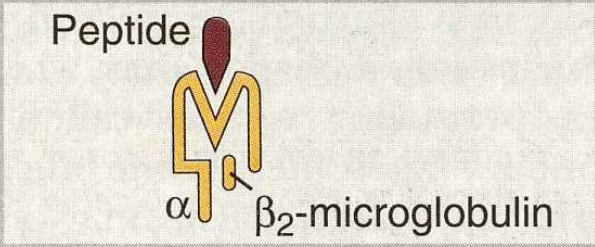
I_i = invariant chain which protects MHC class II from binding to protein

MIIC = MHC class II compartment

MHC

class I

class II

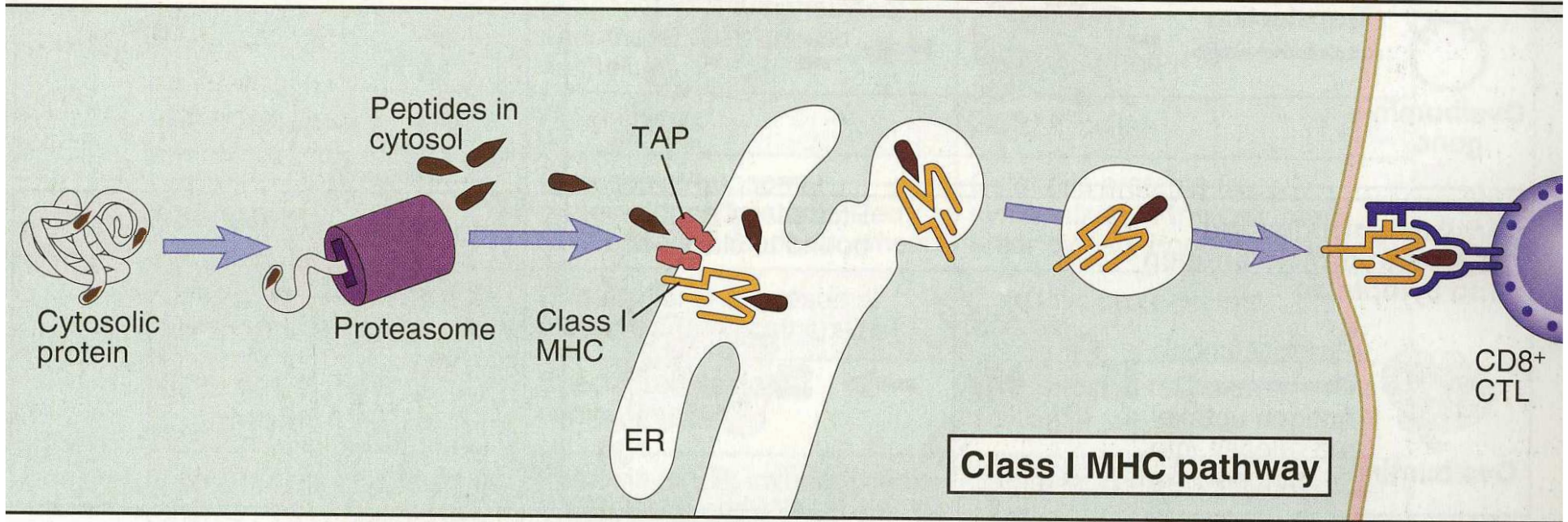
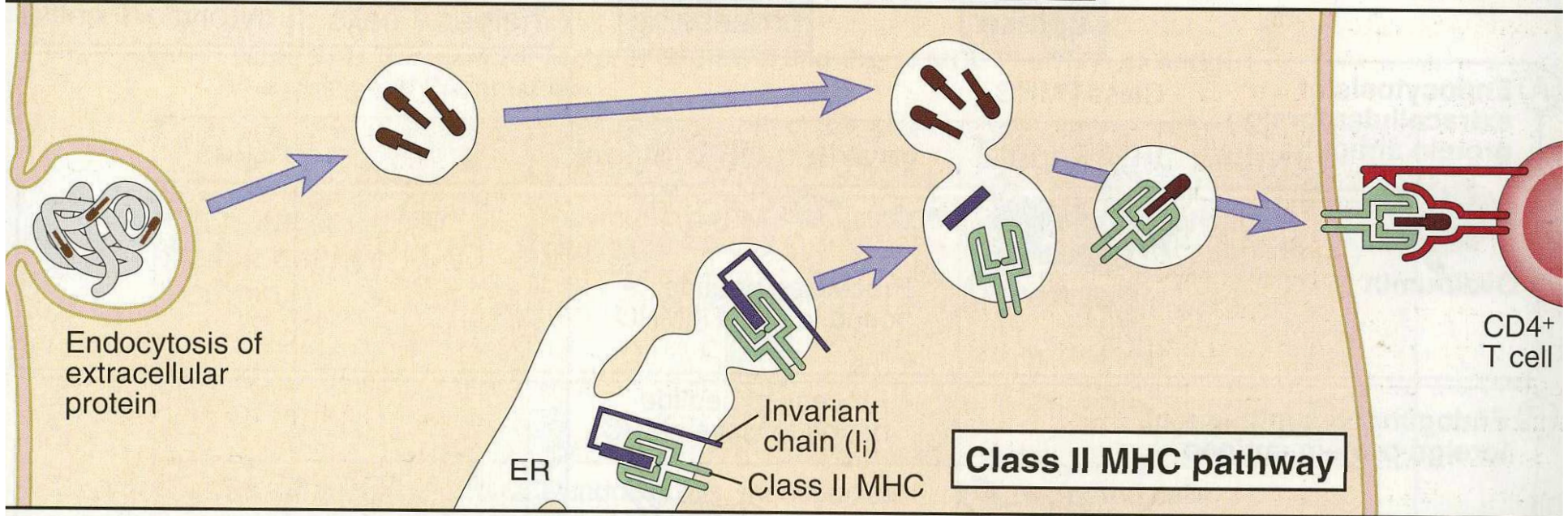
Feature	Class II MHC pathway	Class I MHC pathway
Composition of stable peptide-MHC complex	Polymorphic α and β chains, peptide 	Polymorphic α chain, β_2 -microglobulin, peptide 
Types of APCs	Dendritic cells, mononuclear phagocytes, B lymphocytes; endothelial cells, thymic epithelium	All nucleated cells
Responsive T cells	CD4 ⁺ T cells	CD8 ⁺ T cells
Source of protein antigens	Endosomal/lysosomal proteins (mostly internalized from extracellular environment)	Cytosolic proteins (mostly synthesized in the cell; may enter cytosol from phagosomes)
Enzymes responsible for peptide generation	Endosomal and lysosomal proteases (e.g., cathepsins)	Cytosolic proteasome
Site of peptide loading of MHC	Specialized vesicular compartment	Endoplasmic reticulum
Molecules involved in transport of peptides and loading of MHC molecules	Calnexin in ER; invariant chain in ER, Golgi and MIIC/CIIV; DM	Calnexin, calreticulin, TAP in ER

Antigen uptake

Antigen processing

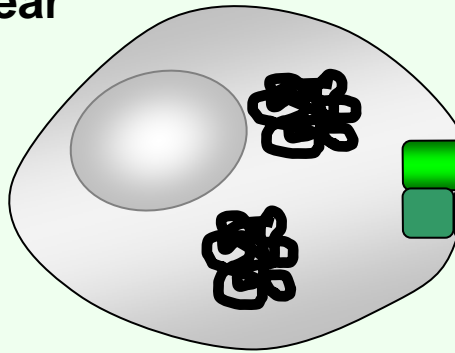
MHC biosynthesis

Peptide-MHC association

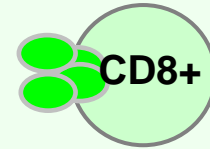
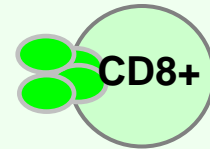
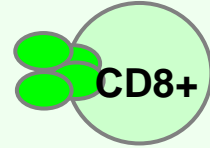
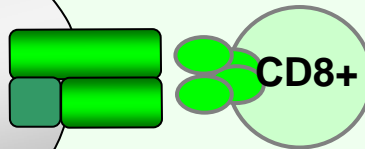


MHC I

nuclear cell



Killing the cell

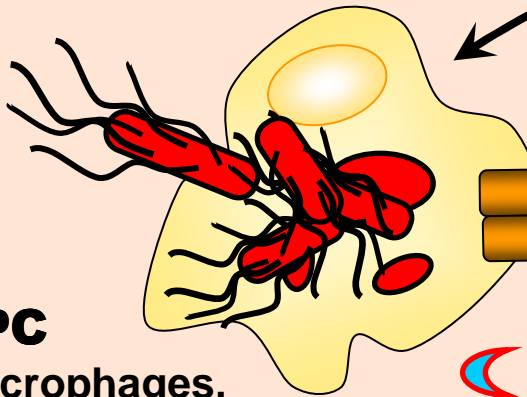


CD8+ T_c lymphocytes
cytotoxic

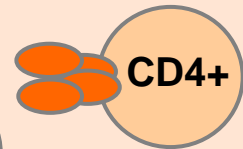
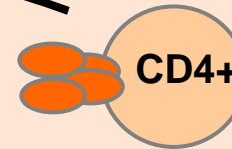
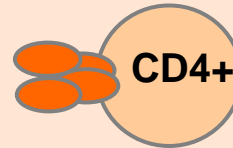
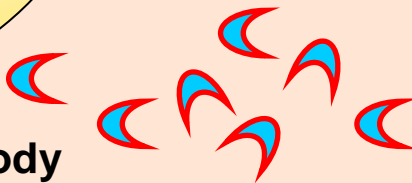
MHC II

Stimulation of phagocytosis

APC
macrophages,
dendritic cell



Antibody
production

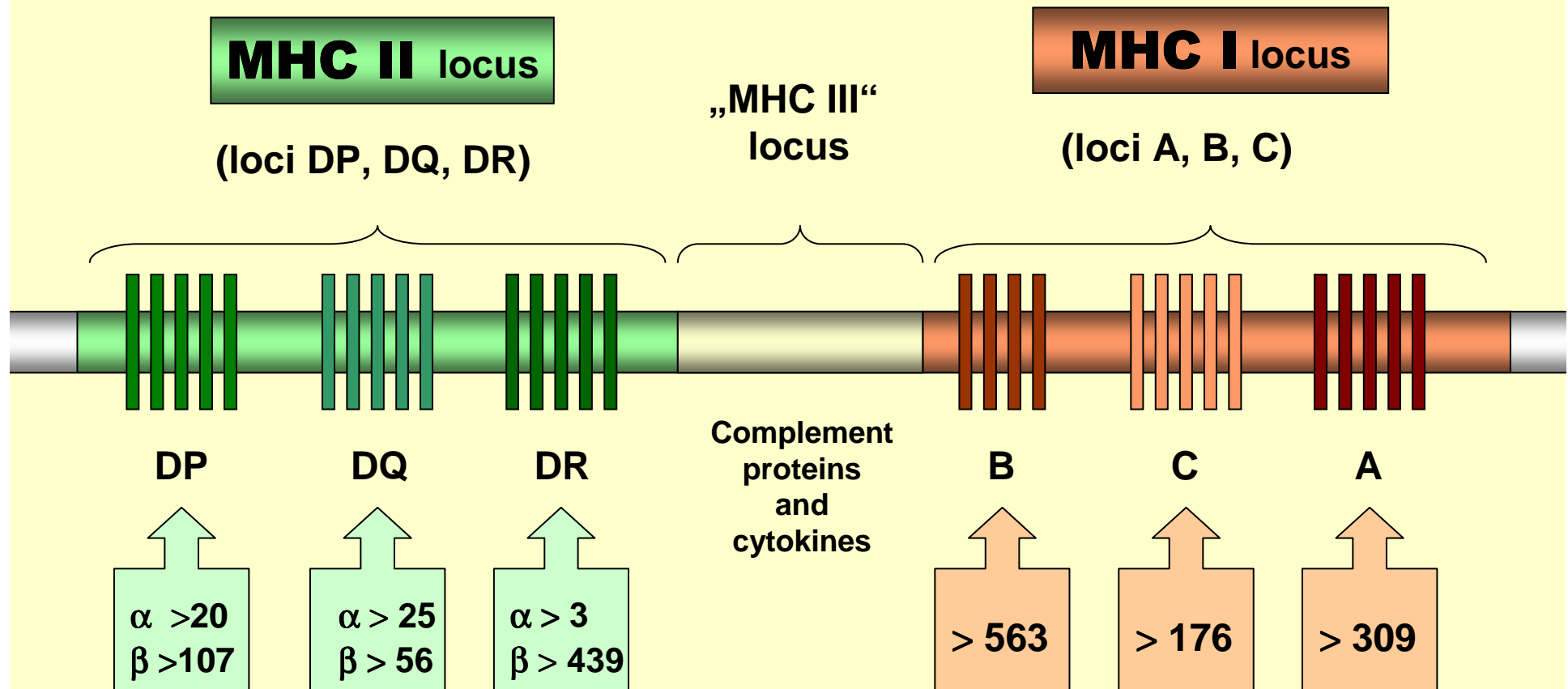


CD4+ T_h lymphocytes
„helper“

**Genes encoding
MHC (HLA) glycoproteins
are the most
polymorphic genes
IN MAMMALIAN GENOME**

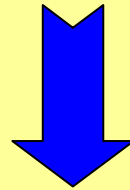
**Located on
the short arm of chromosome 6
in humans**

Structure of the human MHC genes



There are many **DNA variants** [alleles] at each locus and hundreds of **antigenic variants** of MHC molecules [expressed MHC proteins]

**MHC class I and MHC class II from the particular loci
were distinguished using specific antibodies
which could recognize polymorphic variants at the loci**



Serological specificities

**Using newer antibodies
more and more variants of MHC genes
will be found**

Several sequences (genetic variants) can have the same

Serological specificities

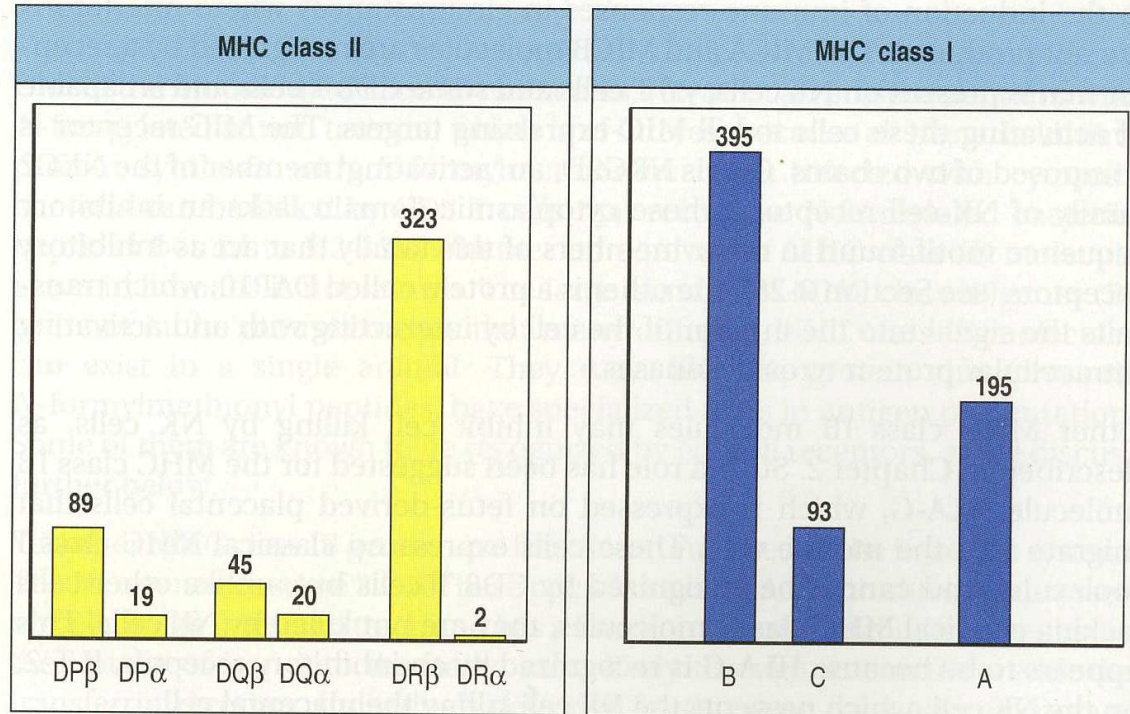
LOCUS	GENETIC VARIANT (sequences)	ANTIBODY USED for detection
HLA-B	HLA-B*1301 HLA-B*1302 HLA-B*1303 HLA-B*1304 HLA-B*1305 HLA-B*1306	HLA-B13

Locus ← **HLA-B*1306** → **Sequence number**

Two digits indicate the serological specificities

Variation of aminoacid sequences change the shape of the binding groove

Human MHC genes are highly polymorphic



August 2000

(Nomenclature Committee
for Factors of HLA system)

Source:

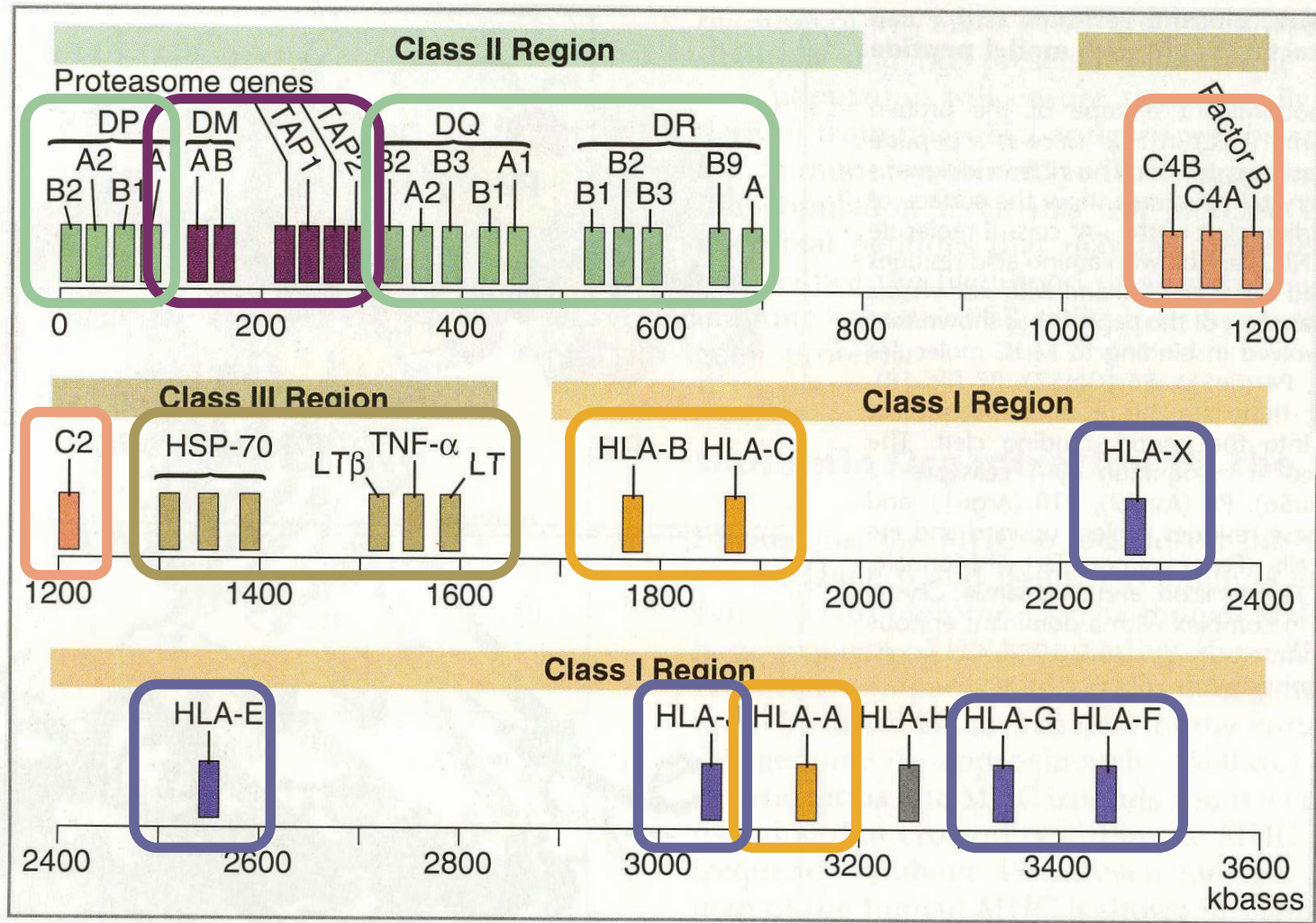
CH. A. Janeway & all.:
IMMUNOBIOLOGY,
the immune system in health
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5th edition, 2001

April 2008: Full list of HLA alleles

HLA class I : <http://www.anthonynolan.org.uk/HIG/lists/class1list.html>

HLA class II : <http://www.anthonynolan.org.uk/HIG/lists/class2list.html>



Genes coded MHC class II molecules

Genes coded complement proteins

Genes coded proteasomes (proteins involved in antigen processing and presentation)

Class I molecules

Class I-like molecules

Genes induced in response to cellular stress (heat shock proteins)

Only identical twins will inherit
Exactly the same set of MHC molecules

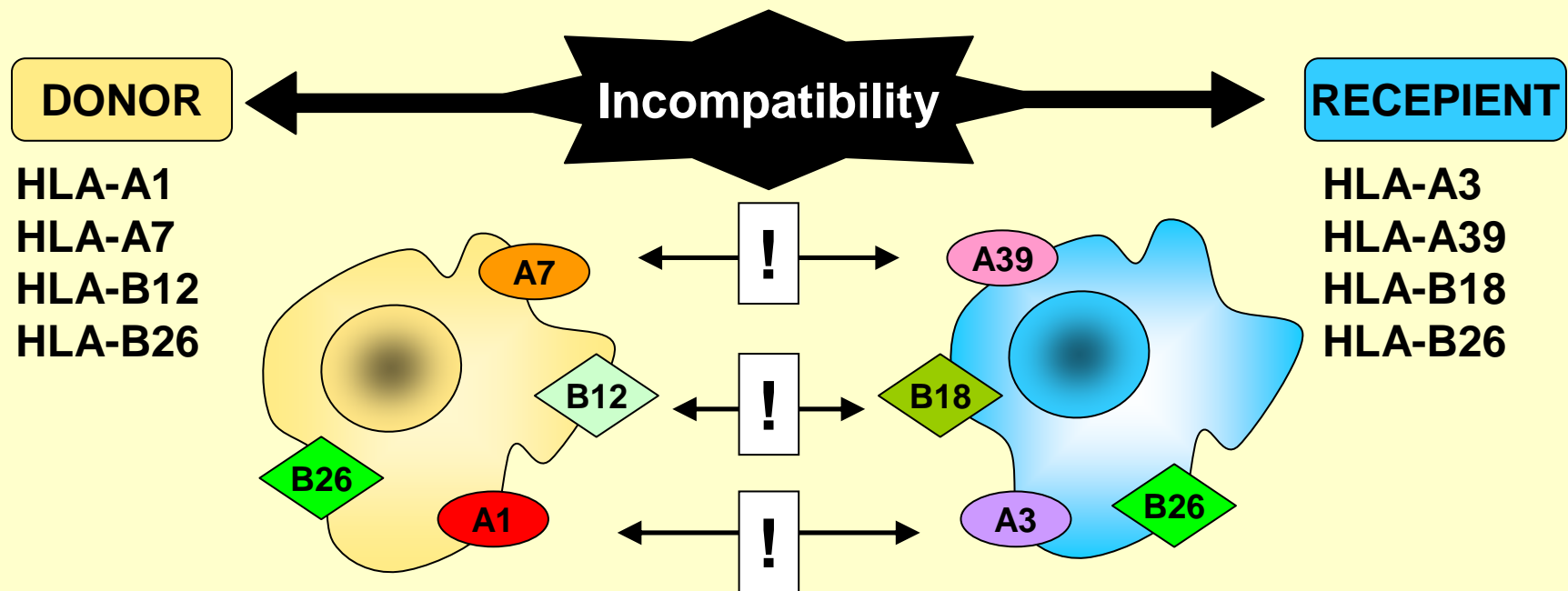
MHC molecules are encoded
by huge numbers of alleles

It is unlikely
that any two people
will inherit
exactly the same set

The repertoire
of MHC proteins
vary
from person to person

MHC molecules are involved in **TRANSPLANT REJECTION**

Foreign MHC molecules of a donor are recognized by
the T cells of recipient



Transplant vocabulary

TRANSPLANTATION is the process of taking cells, tissues or organs called CRAFT, from the DONOR and placing them into a RECIPIENT

❑ AUTOLOGOUS graft (AUTOGRAFT)

A graft transplanted from one individual to the same individual

❑ SYNGENIC graft

A graft transplanted between two genetically identical individuals

❑ ALLOGENIC graft (ALLOGRAFT)

A graft transplanted between two genetically different individuals

❑ XENOGENEIC graft (XENORRAFT)

A graft transplanted between two different species

The molecules that are recognized as foreign on allograft are called

alloantigens

**Alloantigens elicit both
cell-mediate immunity
and
humoral immune response**

Recognition of transplanted cells as

self or **foreign**

is determined by polymorphic genes
that are inherited from both parents and
are expressed codominantly

Codominant expression means that an $(A \times B)F_1$ animal expressed both A and B alleles

Allogenic MHC molecules are presented for recognition in two different ways:

□ DIRECT presentation

Foreign MHC molecule with a bound peptide activate self (recipient) T cell directly.

Allogeneic MHC molecule with a bound peptide can mimic the determinant formed by self MHC molecule plus a foreign peptide.

□ INDIRECT presentation

Foreign MHC molecule may be processed and presented by recipient APC

Processed foreign MHC are recognized by T cells like conventional foreign antigens

Literature

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<http://www.cehs.siu.edu/fix/medmicro/genimm.htm>

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<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/H/HLA.html>

<http://www.bio.davidson.edu/courses/Immunology/hyperhuman/HHH.html>