MHC and Antigen Presentation

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GSK Course, February 19, 2025

T cells recognize antigens in the form of short peptides bound to molecules encoded within the major histocompatibility gene complex (MHC)

Peptide binding to MHC class I and MHC class II molecules

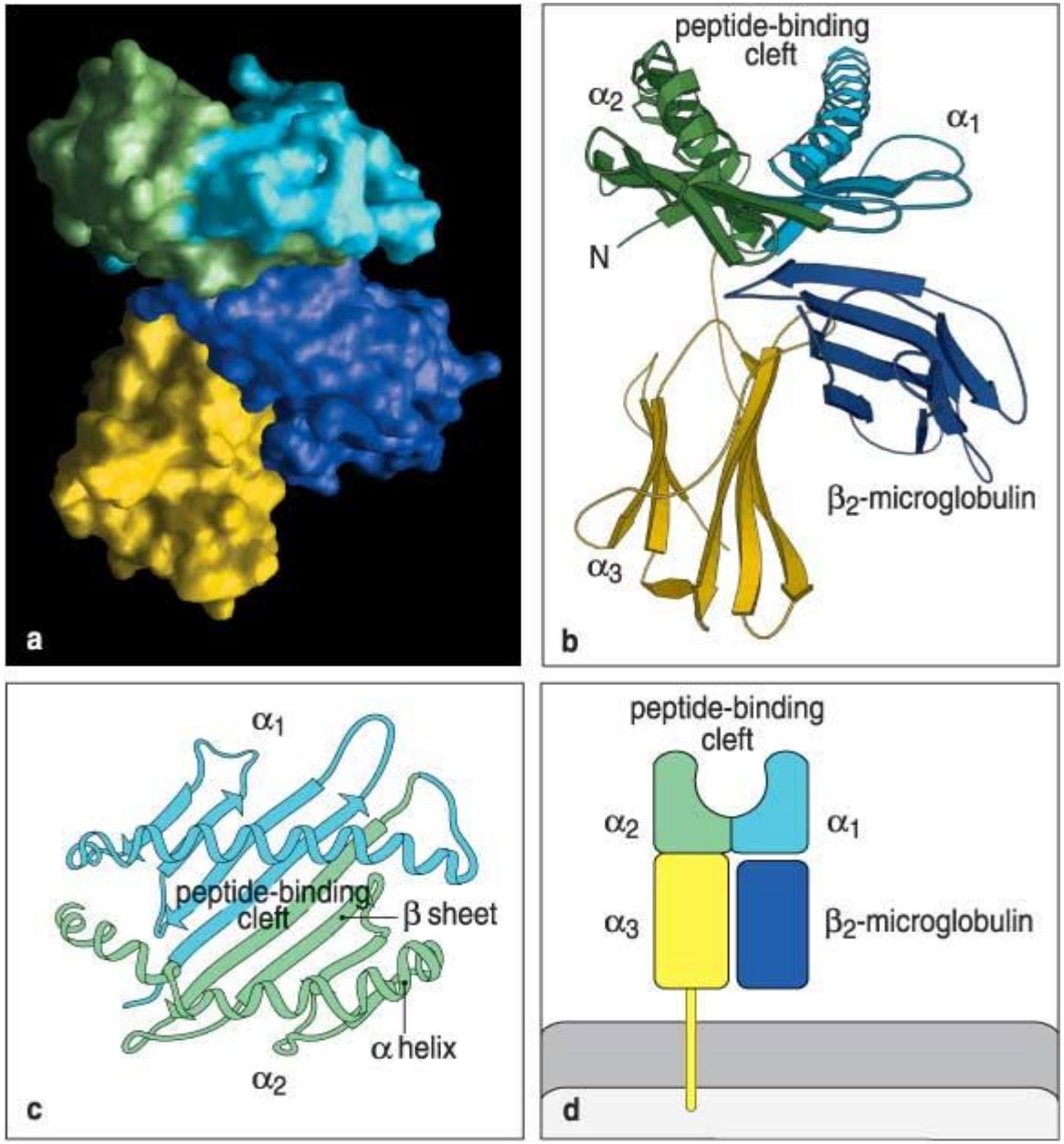
Two major pathways of antigen presentation sample two main sites of protein turnover: cytosol and endo-lysosomes

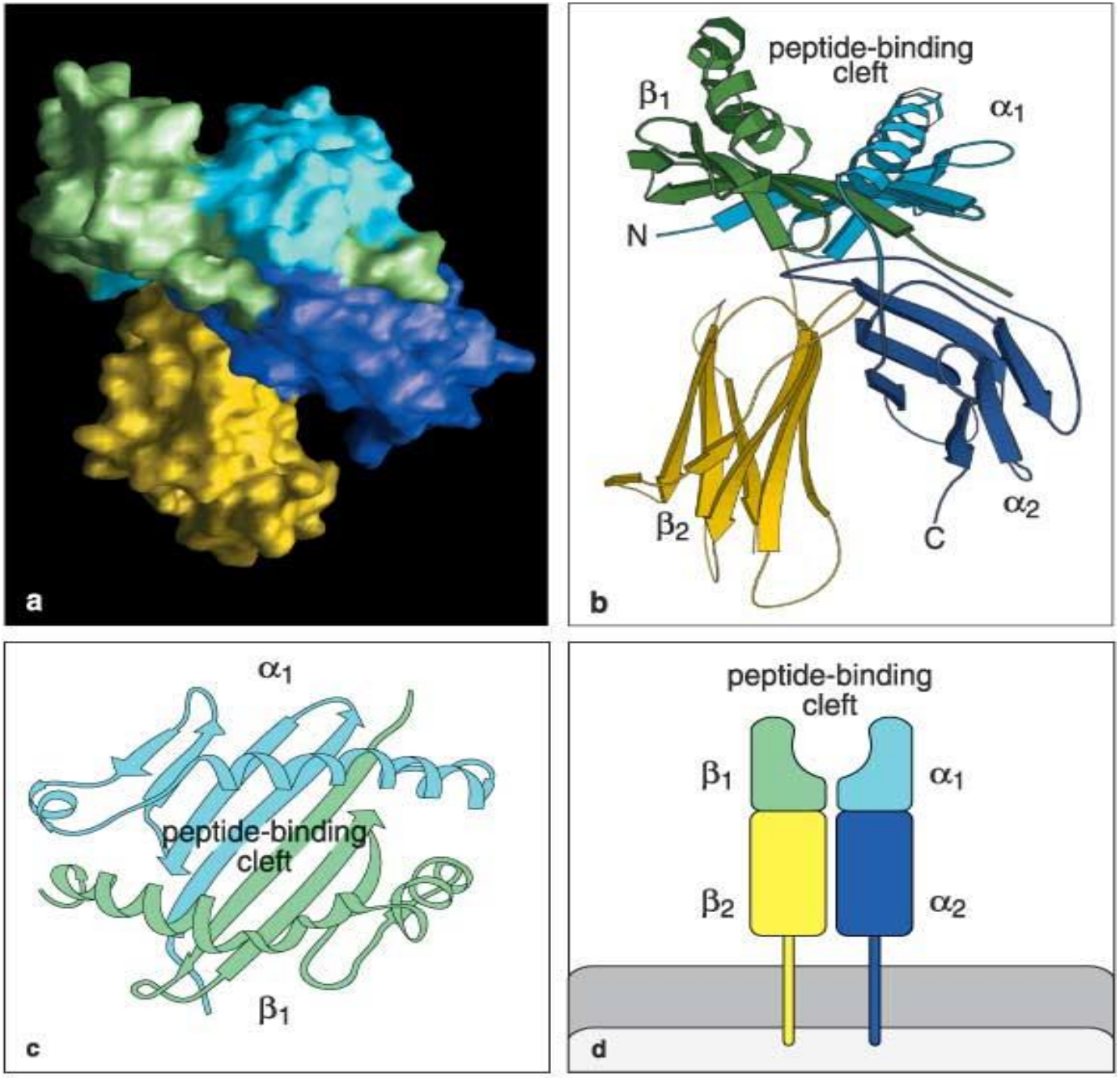
Endosomal antigen presentation by MHC class II molecules

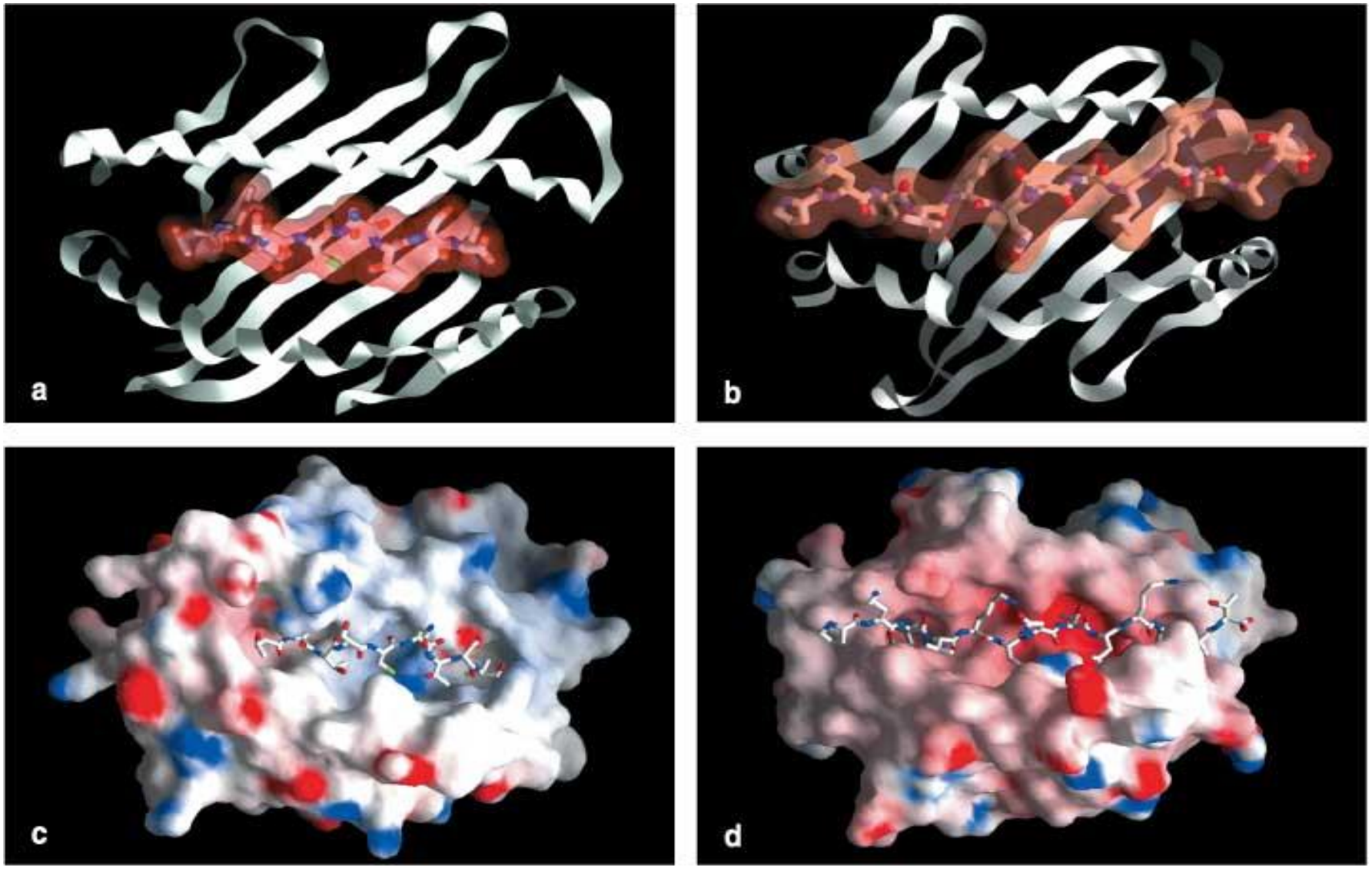
Cytosolic antigen presentation by classical MHC class I molecules

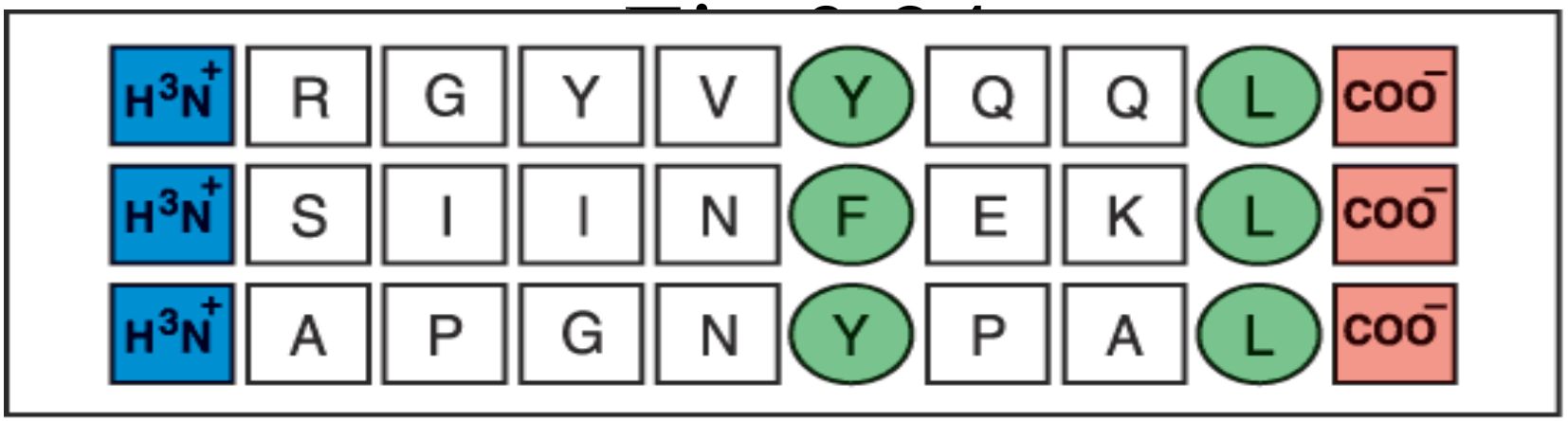
Viral mechanisms to evade antigen presentation by MHC class I molecules

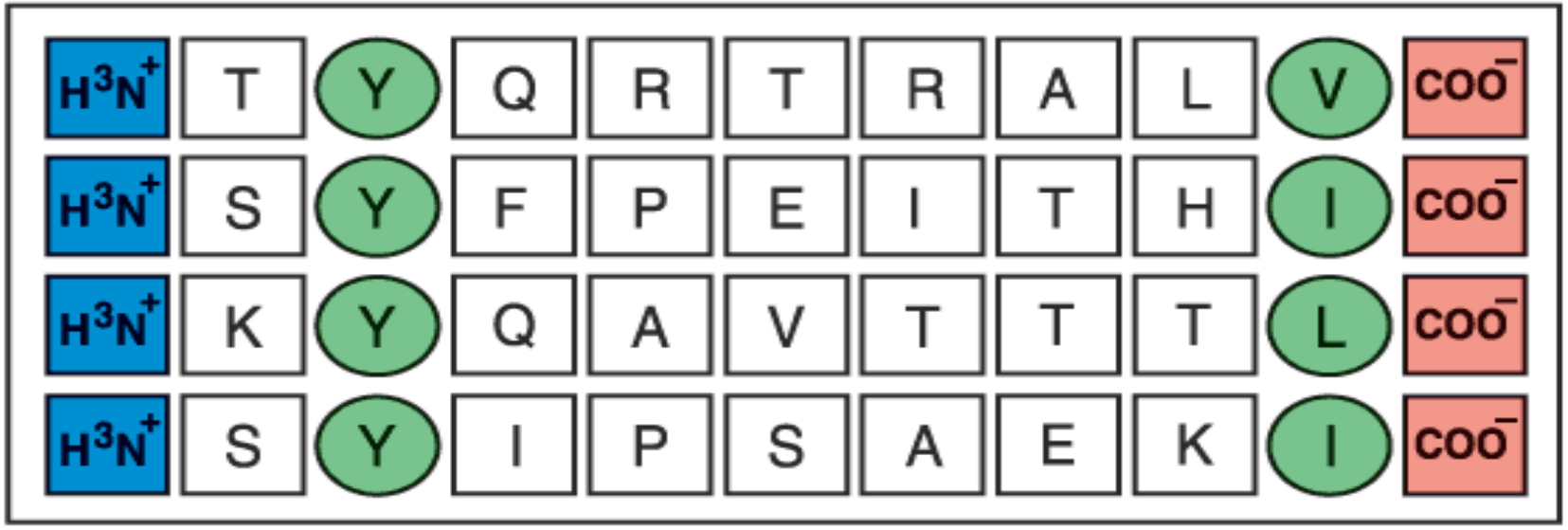
Organization and genetics of MHC locus

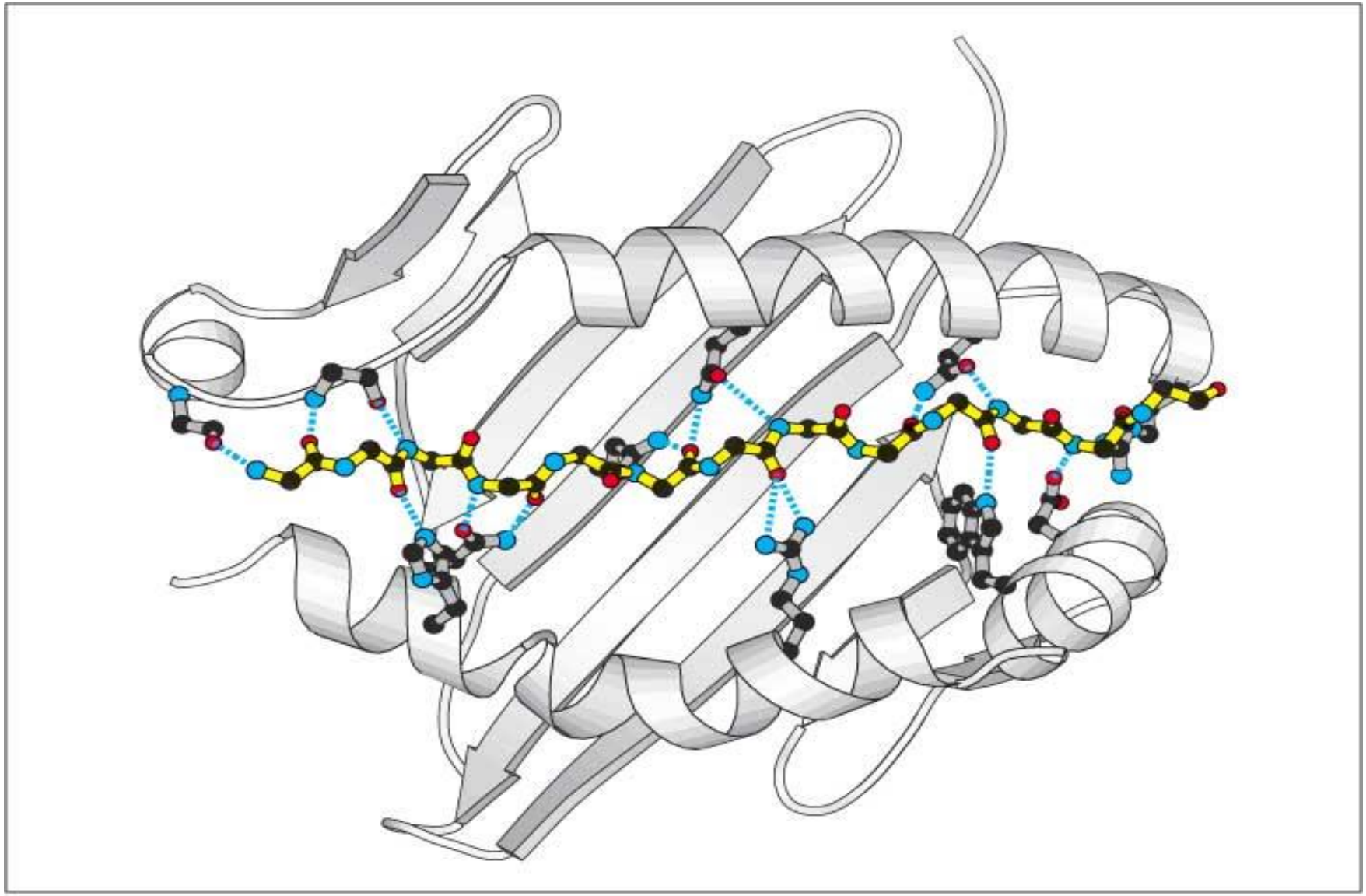

a
peptide-binding cleft
α2
α1
N
β2-microglobulin
α3
b
α1
peptide-binding cleft
β sheet
α helix
α2
c
peptide-binding cleft
α2
α1
α3
β2-microglobulin
d


a
peptide-binding cleft
β1
α1
N
β2
C
α2
b
α1
peptide-binding cleft
β1
c
peptide-binding cleft
β1
α1
β2
α2
d

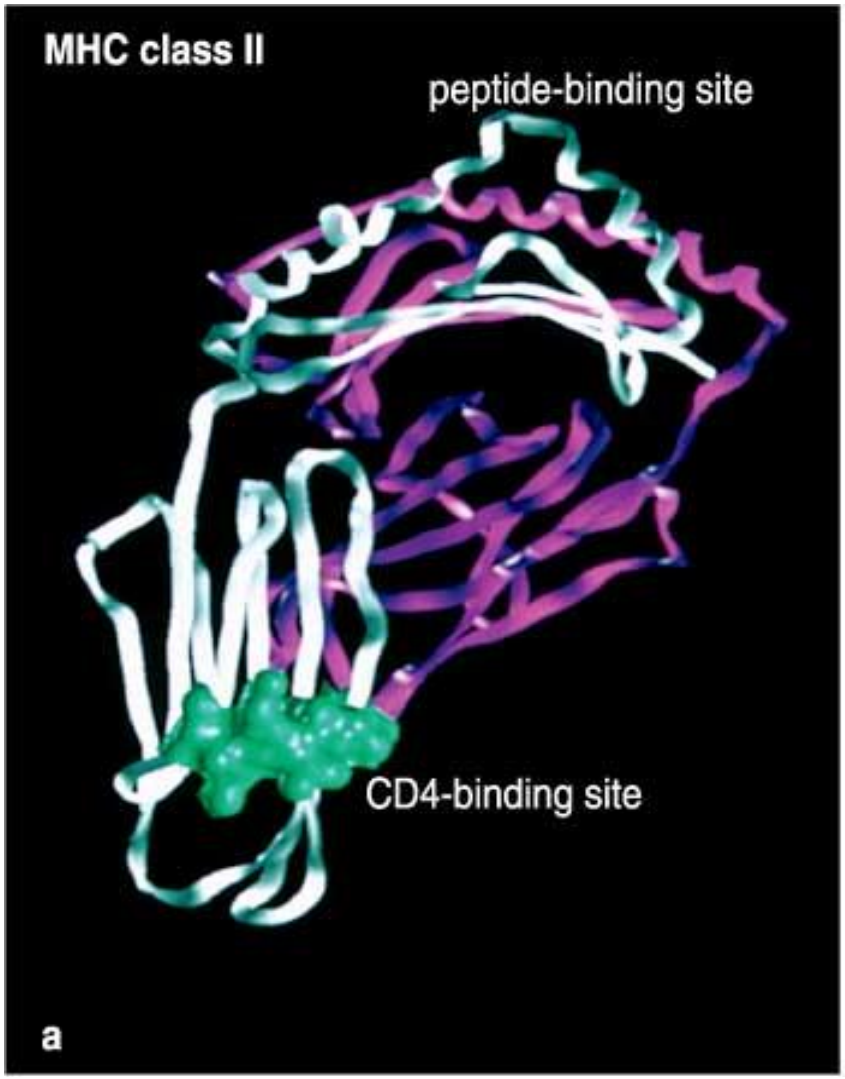

a
b
c
d

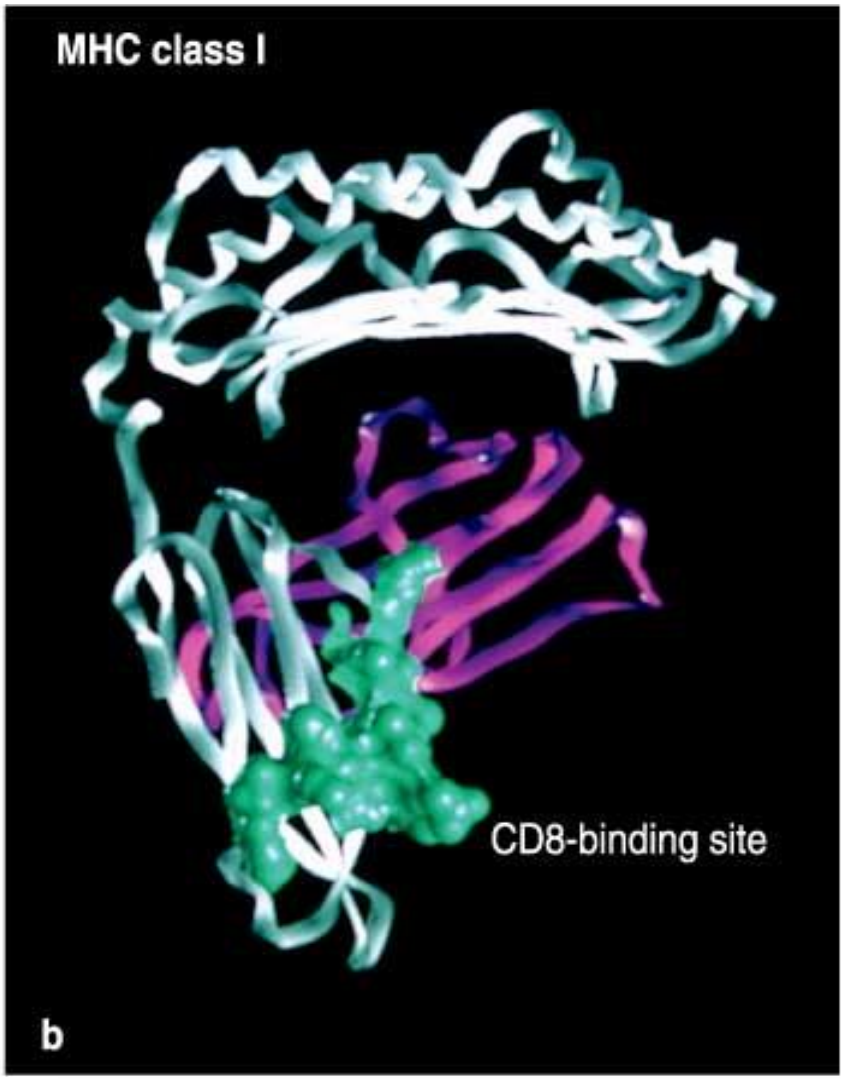

H3N+ R G Y V Y Q Q L COO−
H3N+ S I I N F E K L COO−
H3N+ A P G N Y P A L COO−


H3N+ T Y Q R T R A L V COO−
H3N+ S Y F P E I T H I COO−
H3N+ K Y Q A V T T T L COO−
H3N+ S Y I P S A E K I COO−



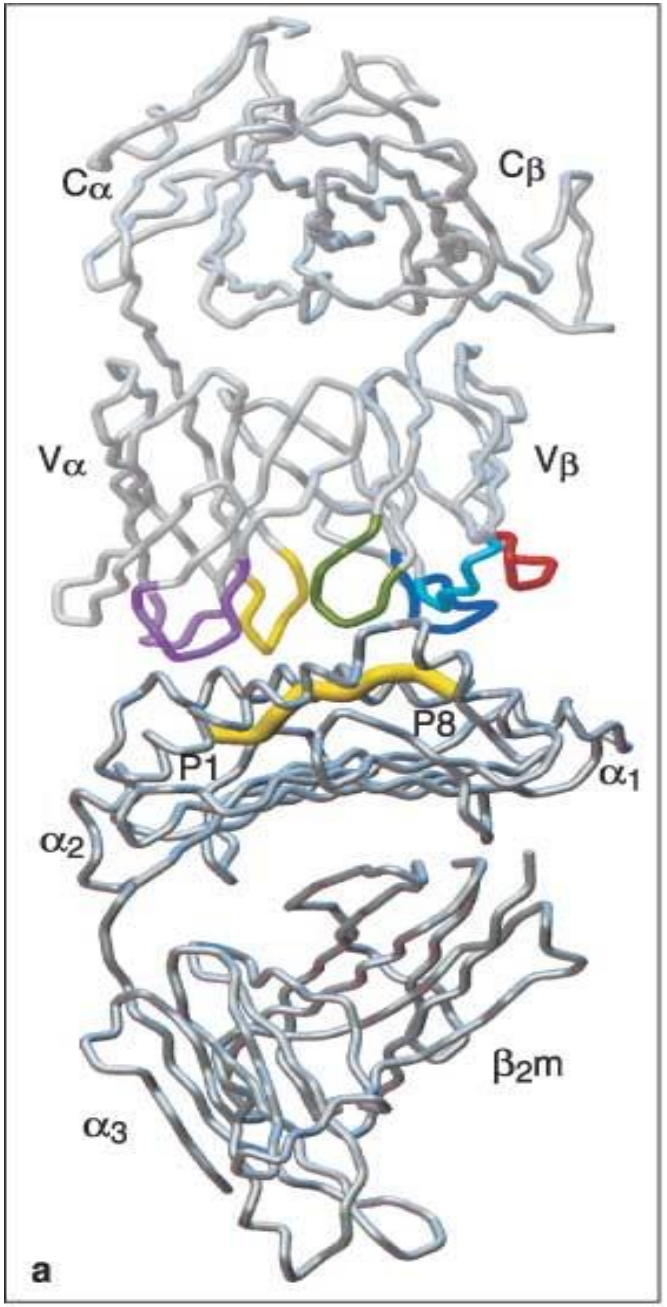
MHC class I	MHC class II
Transmembrane MHC-encoded α chain and soluble β2-m	Transmembrane MHC-encoded α and MHC-encoded β chain
Short cytoplasmic tail	Short cytoplasmic tail
"Closed" groove	"Open" groove
8-10 aa peptides	12-17 aa peptides (or longer)
Ligands for CD8 T cells	Ligands for CD4 T cells
Short-lived	Long-lived

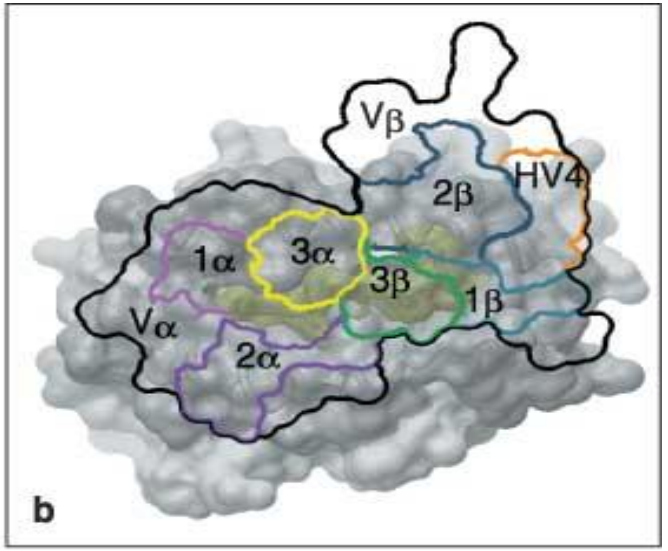




β2 domain

α3 domain


Cα
Cβ
Vα
Vβ
P8
P1
α1
α2
β2m
α3
a


Vβ
HV4
2β
1α
3α
3β
1β
Vα
2α
b

MHC class II presentation of viral and bacterial superantigens

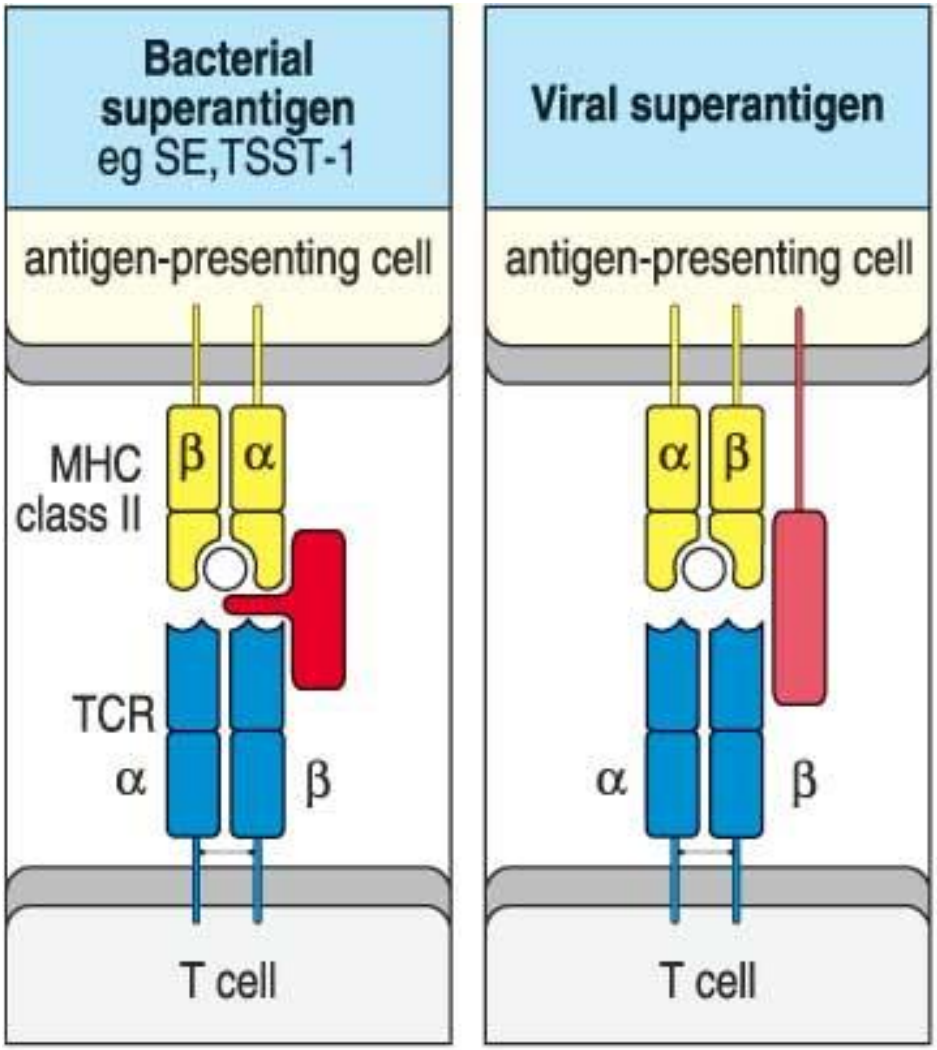
Viral superantigens (vSAG) first discovered as *minor lymphocyte stimulating antigens* or Mls; endogenous mammary tumor viruses

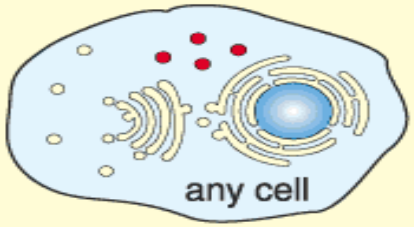
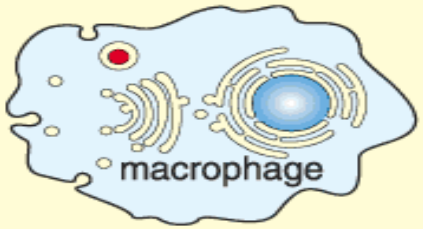
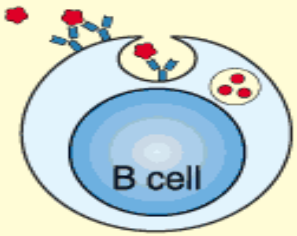
Bacterial superantigens discovered as potent T cell mitogens inducing toxic shock [staphylococcal enterotoxin B and A (SEB and SEA), toxic shock syndrome toxin-1 (TSST-1)]

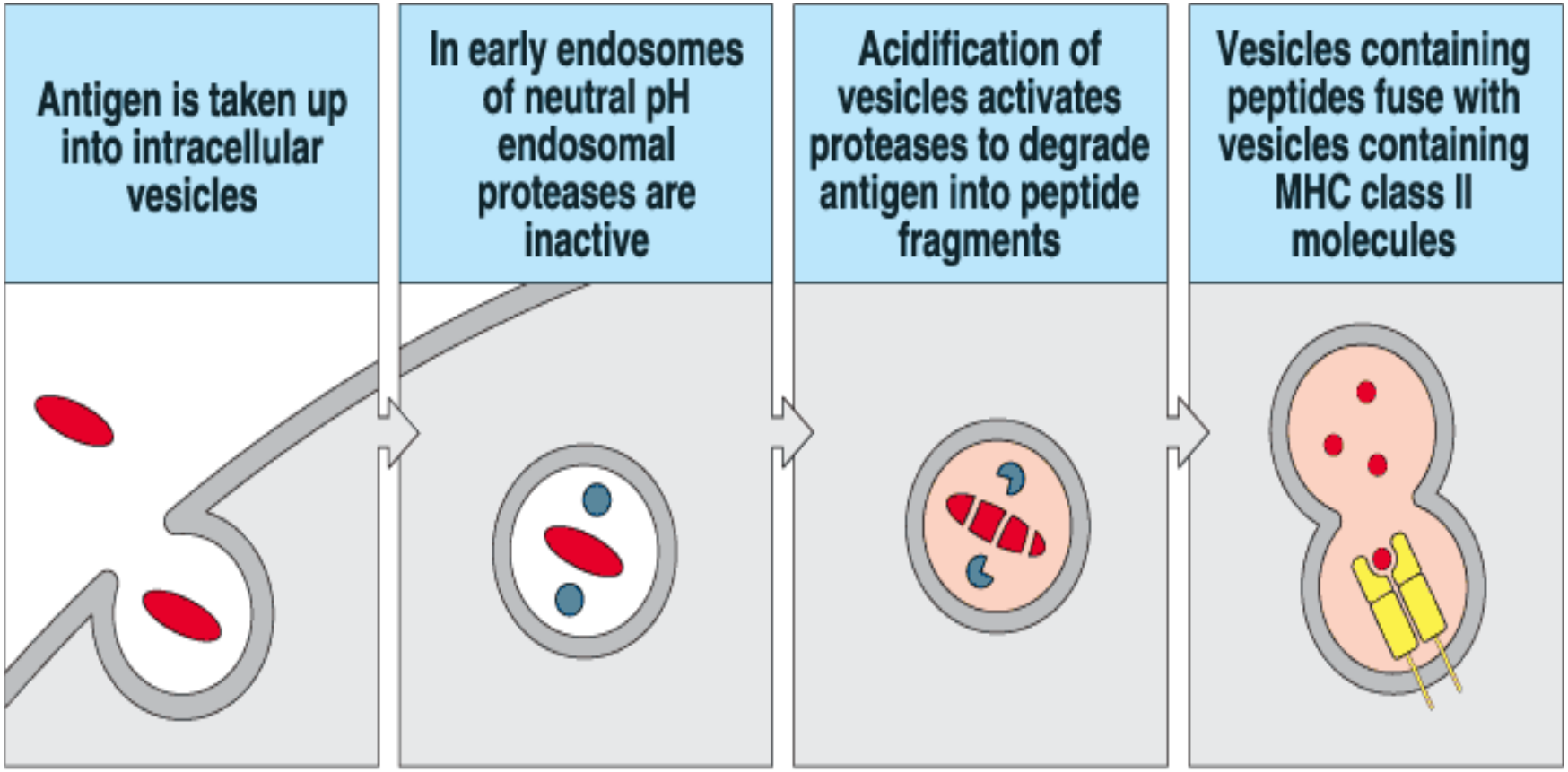
Do not require proteolytic processing

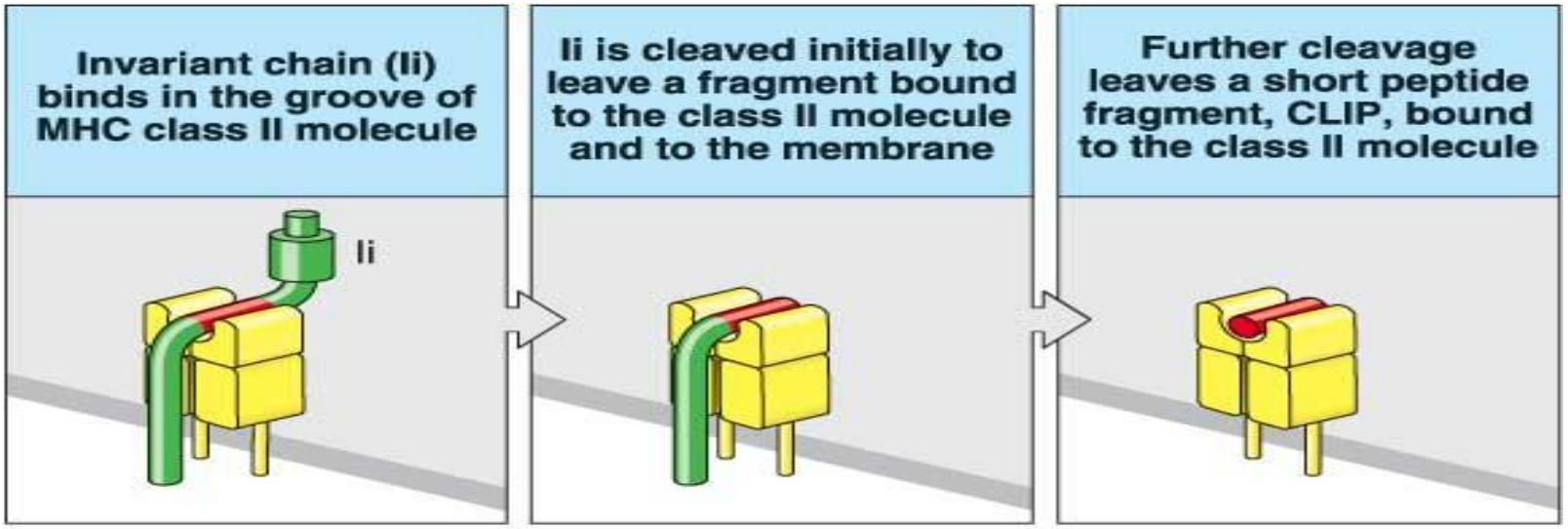
Interact with relatively conserved regions of MHC class II molecules and a particular Vβ element (e.g. Vβ8 or Vβ3)

Activate large number of T cells (all T cells expressing corresponding Vβ).

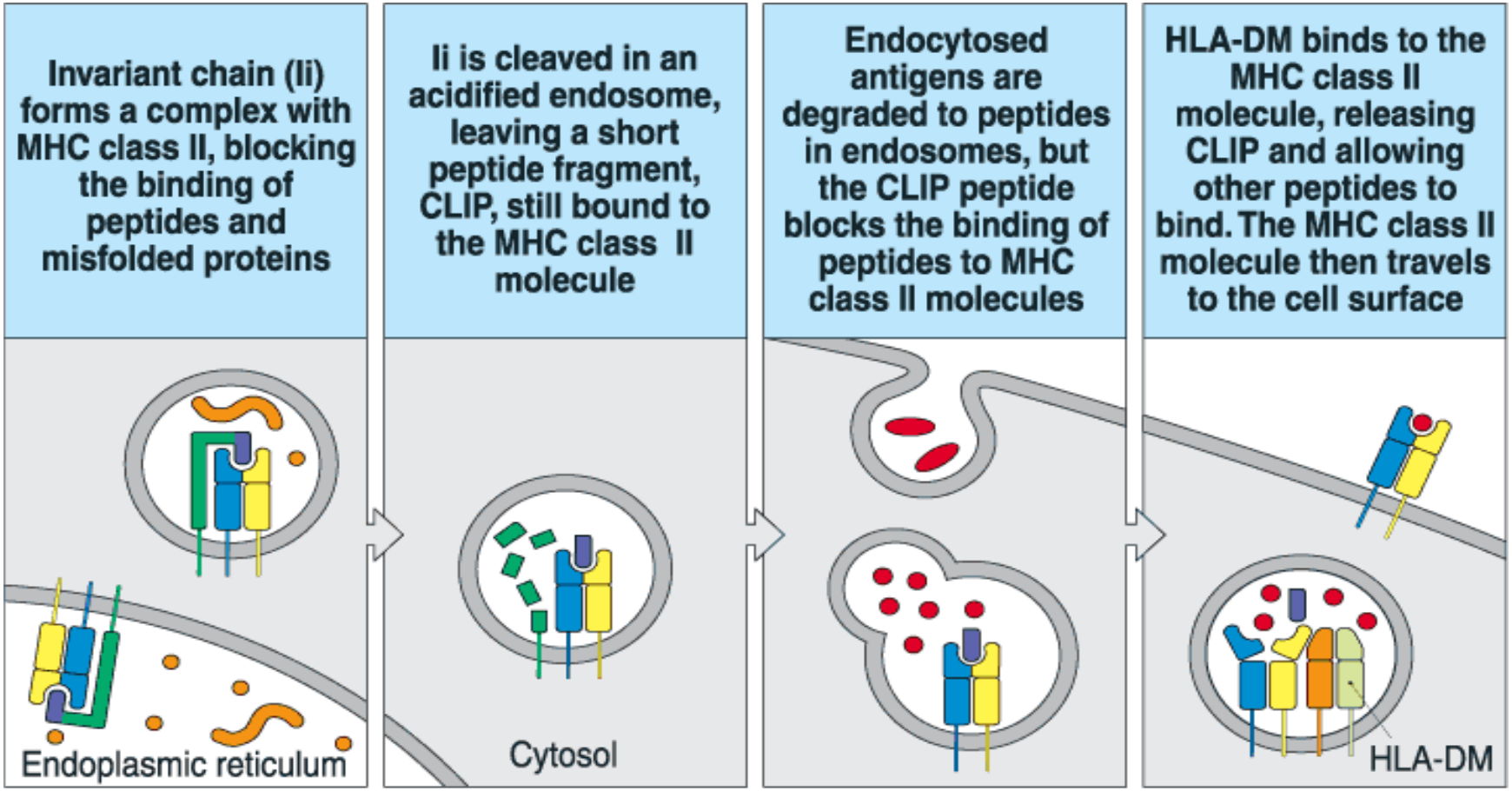

Bacterial superantigen
eg SE,TSST-1
antigen-presenting cell
MHC class II
β
α
TCR
α
β
T cell
Viral superantigen
antigen-presenting cell
α
β
α
β
T cell

	Cytosolic pathogens	Intravesicular pathogens	Extracellular pathogens and toxins
			
Degraded in	Cytosol	Endocytic vesicles (low pH)	Endocytic vesicles (low pH)
Peptides bind to	MHC class I	MHC class II	MHC class II
Presented to	CD8 T cells	CD4 T cells	CD4 T cells
Effect on presenting cell	Cell death	Activation to kill intravesicular bacteria and parasites	Activation of B cells to secrete Ig to eliminate extracellular bacteria/toxins


Antigen is taken up into intracellular vesicles
In early endosomes of neutral pH endosomal proteases are inactive
Acidification of vesicles activates proteases to degrade antigen into peptide fragments
Vesicles containing peptides fuse with vesicles containing MHC class II molecules



p33
(p35)
p41
(p43)


Invariant chain (Ii) forms a complex with MHC class II, blocking the binding of peptides and misfolded proteins
Ii is cleaved in an acidified endosome, leaving a short peptide fragment, CLIP, still bound to the MHC class II molecule
Endocytosed antigens are degraded to peptides in endosomes, but the CLIP peptide blocks the binding of peptides to MHC class II molecules
HLA-DM binds to the MHC class II molecule, releasing CLIP and allowing other peptides to bind. The MHC class II molecule then travels to the cell surface
Endoplasmic reticulum
Cytosol
HLA-DM

HLA-DO/H-2O

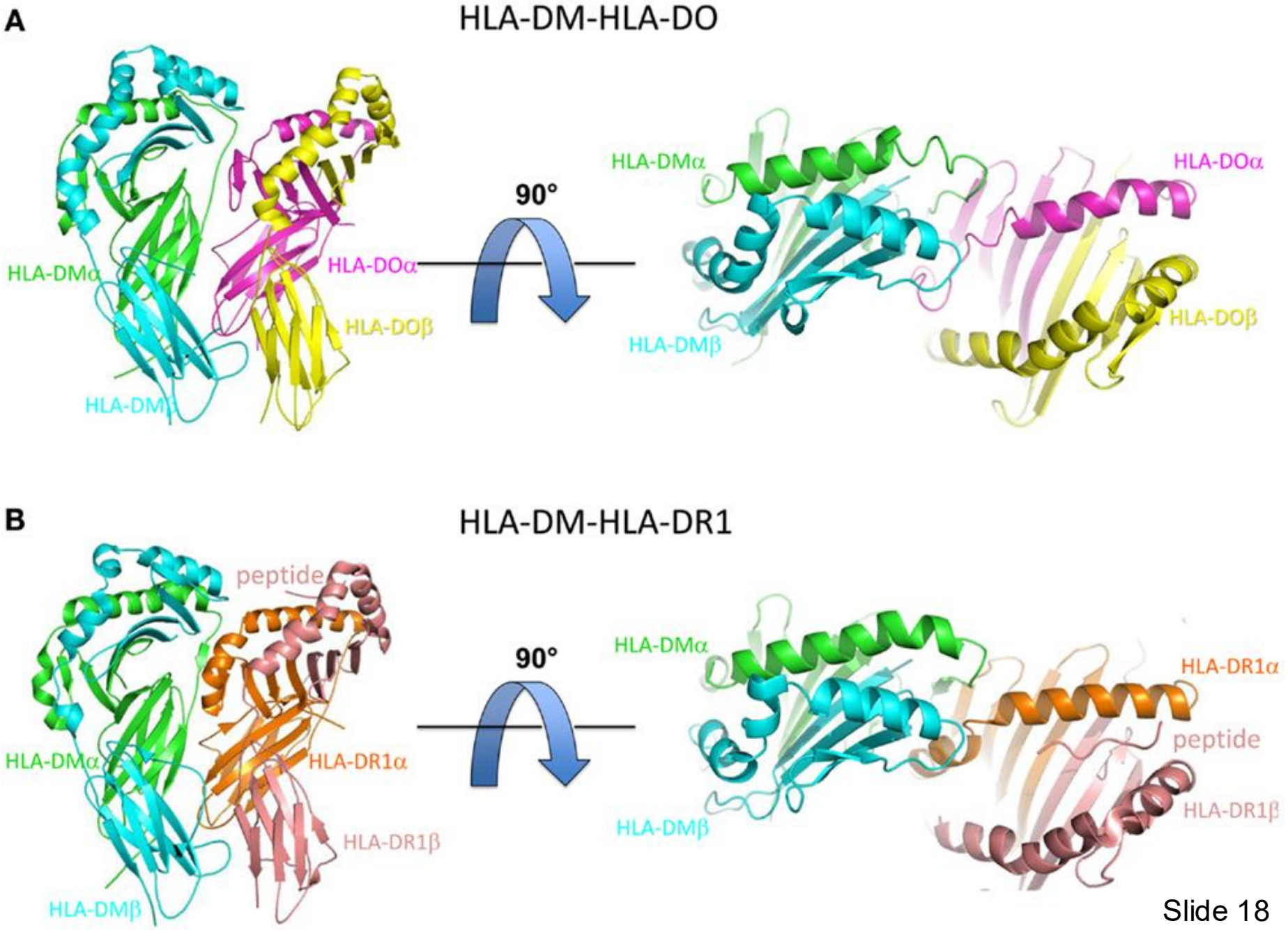
Expressed in B cells and mTEC

Binds DM

Inhibits DM function in a pH-dependent manner

Free DM is localized to internal vesicles of multi-vesicular bodies

DM-DO are localized to the outside membrane


A
HLA-DM-HLA-DO
HLA-DMα
HLA-DOα
HLA-DOβ
HLA-DMβ
90°
HLA-DMα
HLA-DOα
HLA-DOβ
HLA-DMβ
B
HLA-DM-HLA-DR1
peptide
HLA-DMα
HLA-DR1α
HLA-DR1β
HLA-DMβ
90°
HLA-DMα
HLA-DR1α
peptide
HLA-DR1β
HLA-DMβ

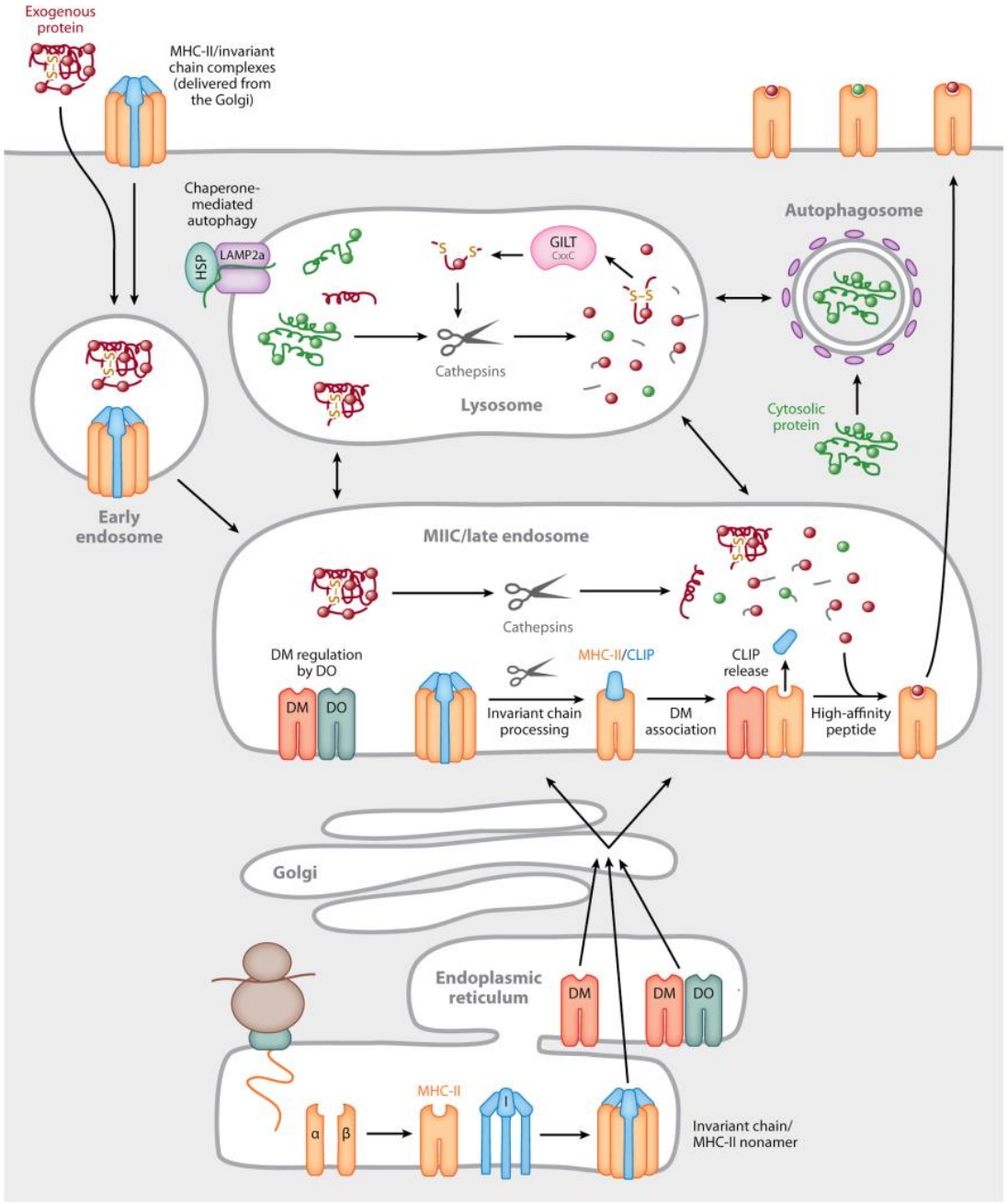
Lysosomal proteases:

AEP/legumain/?
(Manoury et al., 2003)

Cathepsin S and L
(Nakagawa et al., 1998;1999)

SPPL2a
(Schneppenheim et al., 2013;
Beisner et al., 2013)

Lysosomal thioreductases:
GILT
(Maric et al., 2001)



Blum JS, et al. 2013.
Annu. Rev. Immunol. 31:443–73

MHC class I-like CD1 molecules present glycolipids to NKT cells (and CD8 T cells in humans)

CD1d (conserved), CD1b,c,d (human)

Formed by non-MHC encoded α-chain and soluble β2m

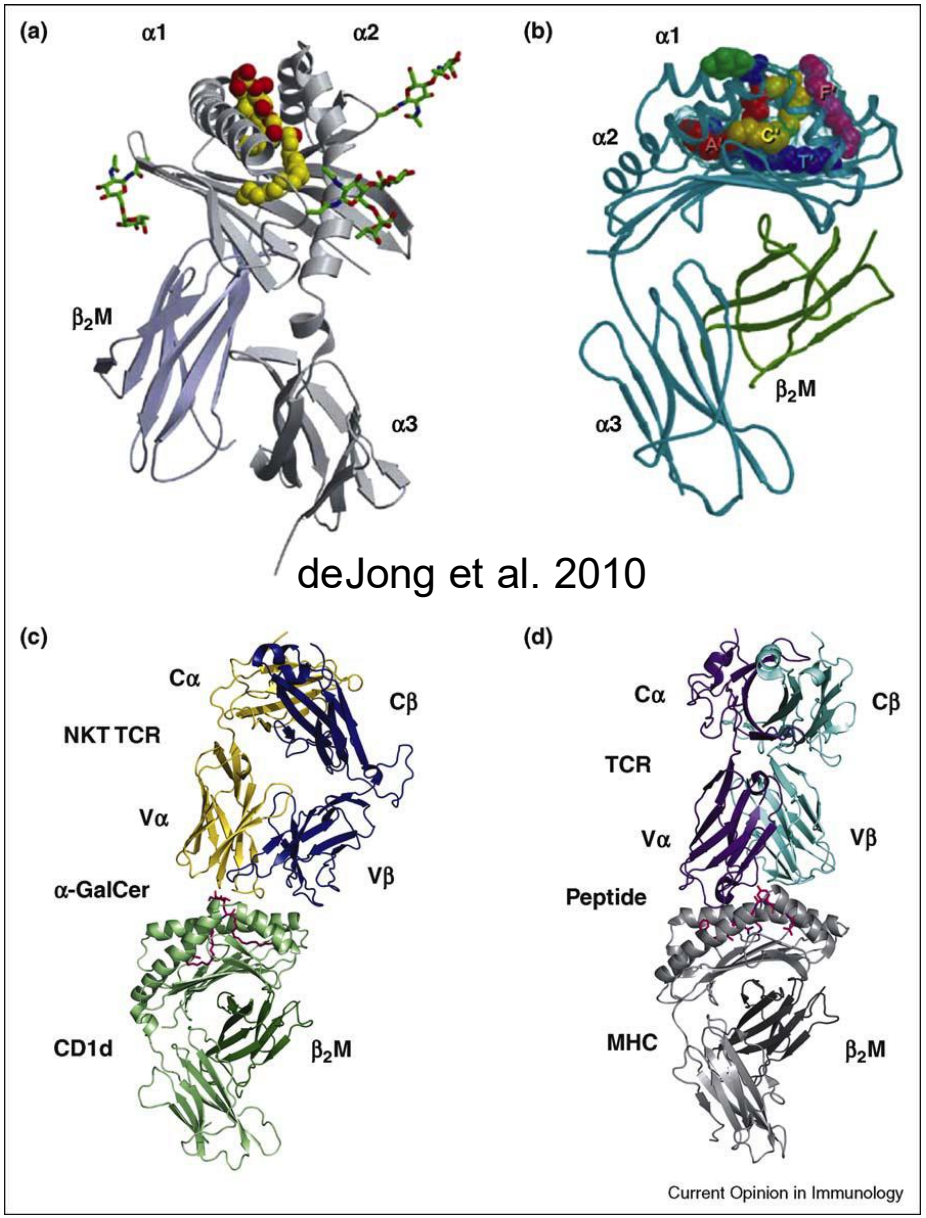
Cytoplasmic tail contains endosomal localization sequence

Expressed by B cells, T cells, dendritic cells, epithelial cells

Present glycolipids in the endosomal/lysosomal compartment (MIIC)

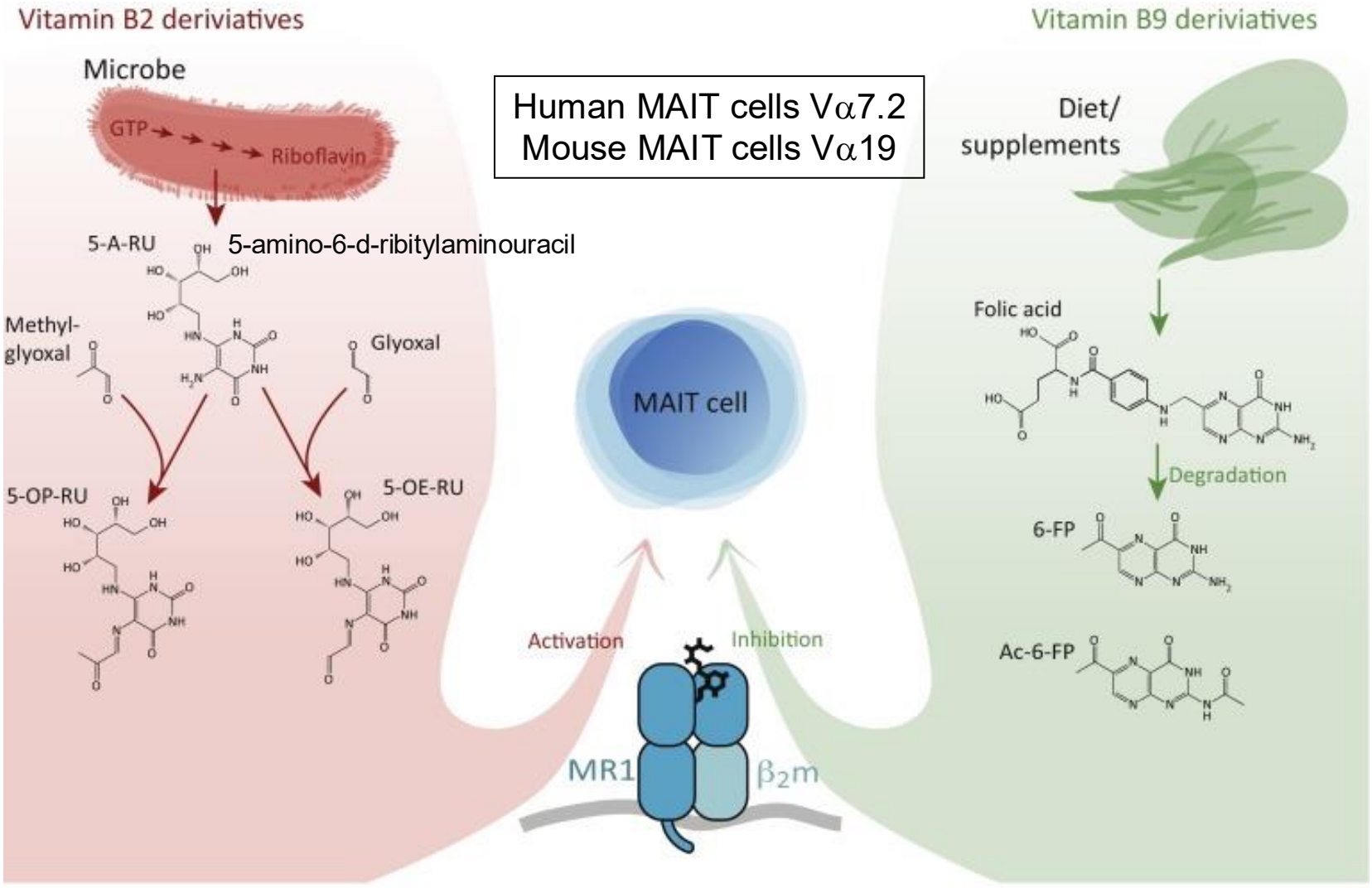
Mycobacterial lipids and lipopeptides, e.g. lipoarabinomannan, mycobactin

Human NKT cells Vα24Jα18
Mouse NKT cells Vα14Jα18



(a) The crystal structure of CD1a in the presence of the self-antigen sulfatide [48]. In green are three N-linked sugar moieties while yellow spheres are ligand (3-O-sulfogalactosylceramide) carbon atoms. (b) CD1b was crystallized in complex with both phosphatidylinositol (PI) and a ganglioside GM2 [66]. (c) The first co-crystal of a CD1 family member with a TCR was generated for human CD1d–a-GalCer in complex with the iNKT TCR revealing a novel binding mode that had not previously been seen with classical MHC class I-restricted and II-restricted TCR [62]. (d) HLA-B8–peptide–TCR (LC13) complex

MR-1 presents vitamin B2 and B9 biosynthesis derivatives to MAIT cells



5-(2-oxopropylideneamino)-6-d-ribitylaminouracil (5-OP-RU)

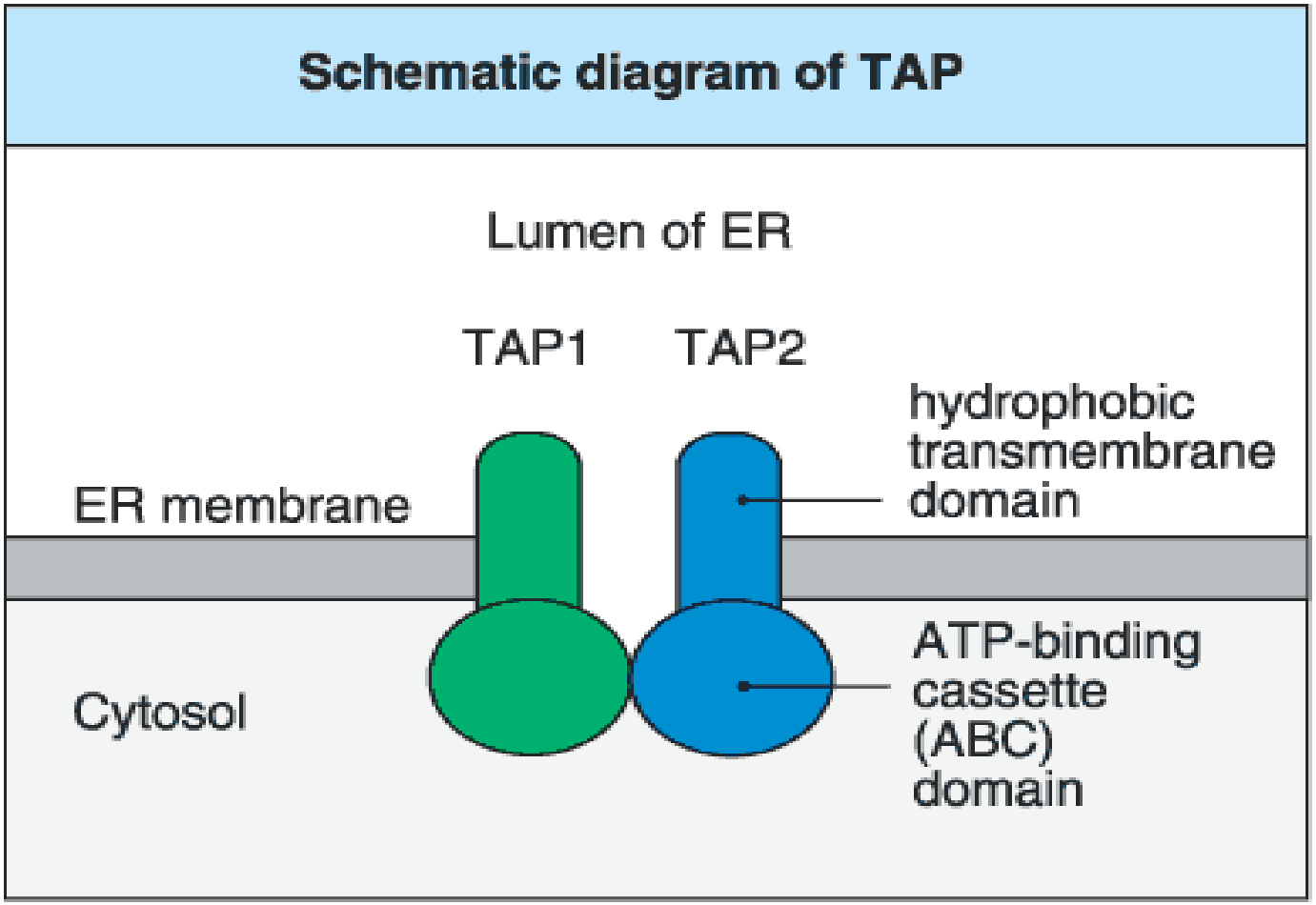
5-(2-oxoethylideneamino)-6-d-ribitylaminouracil (5-EP-RU)

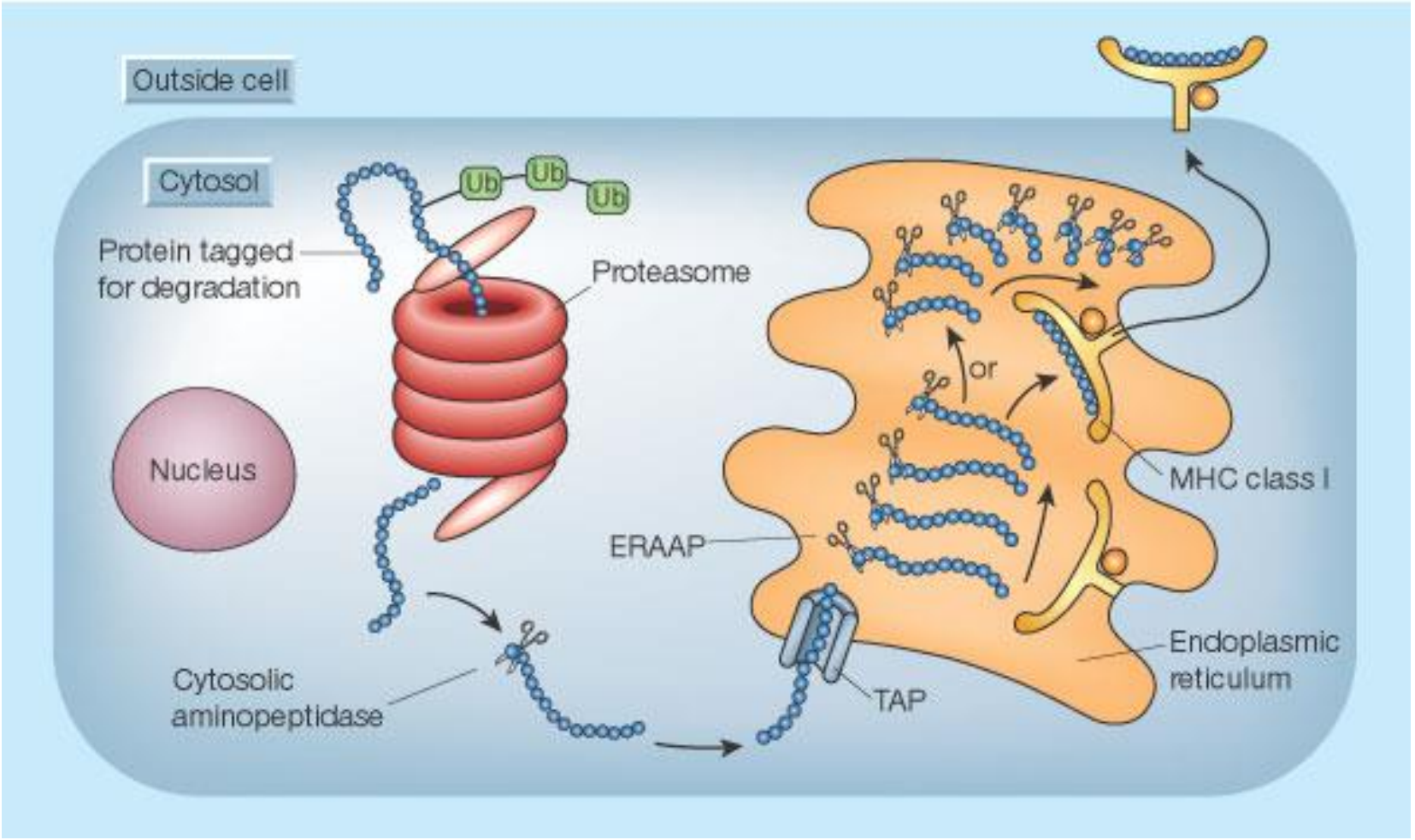
6-formyl-pterin

acetyl-6-formyl-pterin

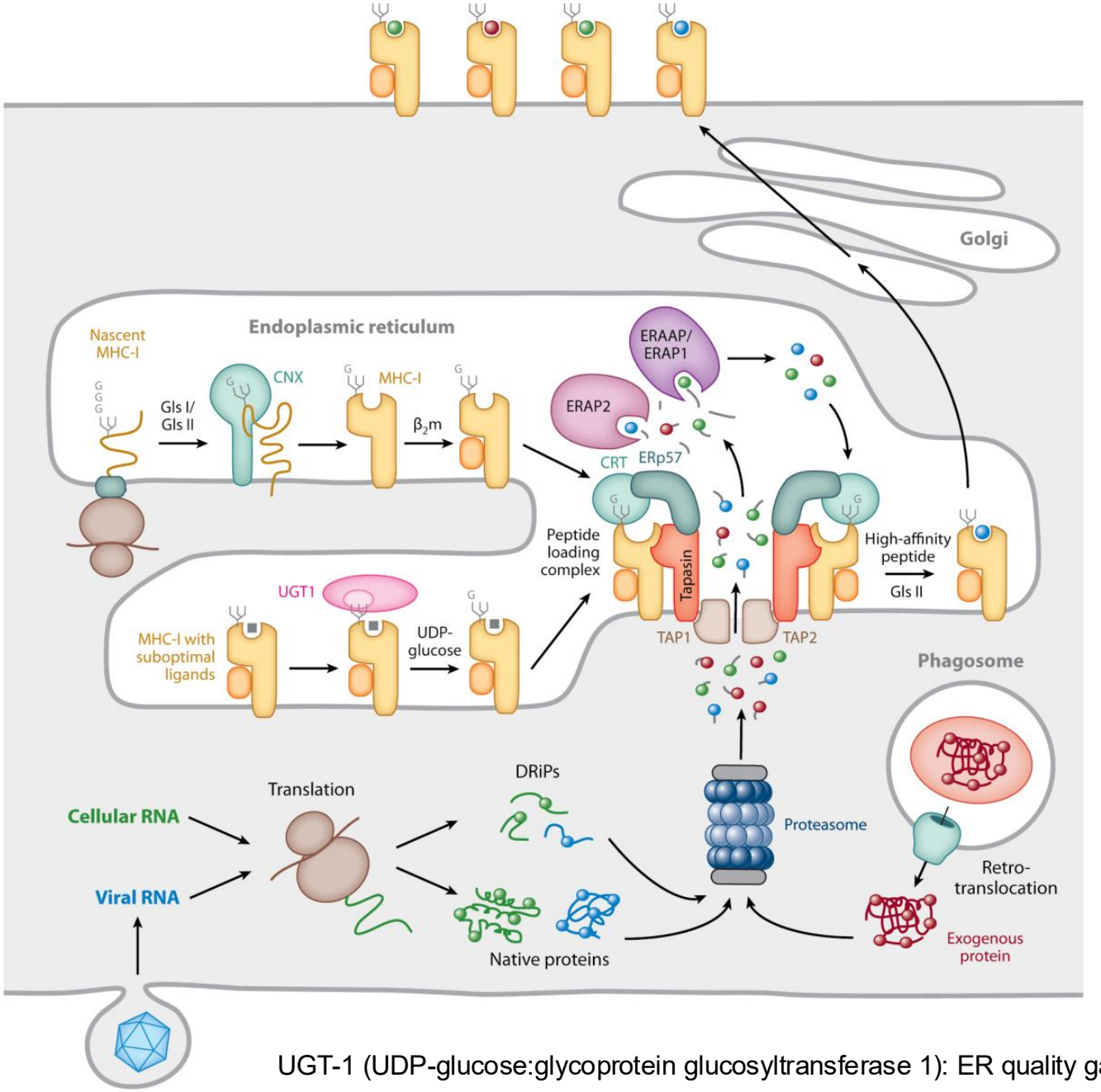
Trends in Immunology

Intracellular assembly of MHC class I peptide complexes


Schematic diagram of TAP
Lumen of ER
TAP1
TAP2
hydrophobic transmembrane domain
ER membrane
Cytosol
ATP-binding cassette (ABC) domain



26S proteosome (20S proteolytic core + 19S regulatory complexes)
constitutive and inducible subunits of immunoproteosome (LMP2, LMP7 and MECL-1, PA28)
ERAAP ER aminopeptidase associated with antigen processing



UGT-1 (UDP-glucose:glycoprotein glucosyltransferase 1): ER quality gatekeeper

Gls I/II – alpha-glucosidase I/II

Blum JS, et al. 2013.
Annu. Rev. Immunol. 31:443–73

MHC class I Antigen Presentation of Cytosolic Peptides

Protein degradation in the cytosol

Proteosomes - multicatalytic protease complex involved in degradation of ubiquitin-marked proteins

Two types of proteosomes with constitutive and inducible subunits (LMP2, LMP7 and MECL-1, regulator PA28)

Peptide Transport

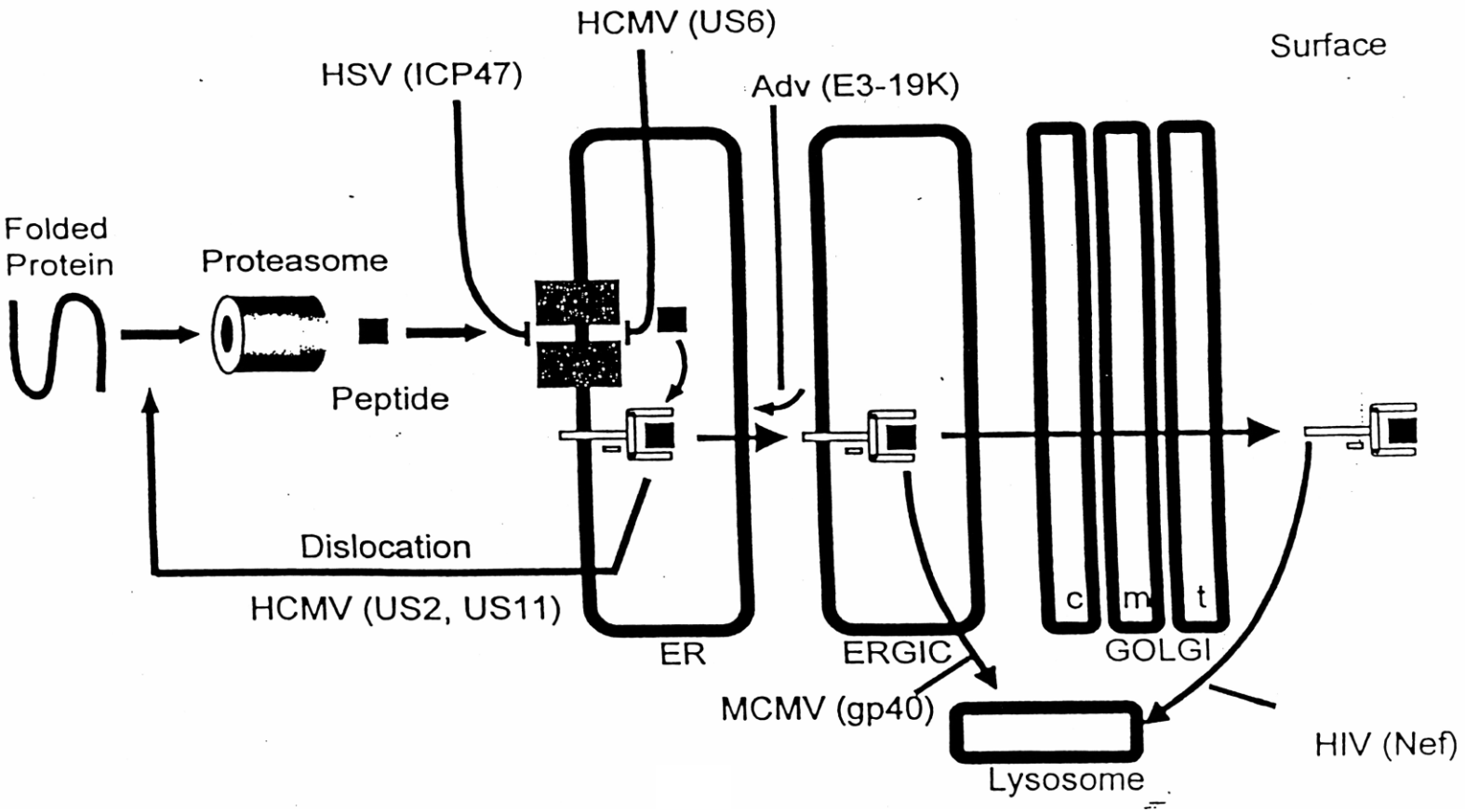
TAP-1/TAP-2 (transporters associated with antigen presentation) ABC (ATP-Binding Cassette) family members

Molecules involved in MHC class I presentation

Chaperones: calnexin, calreticulin, tapasin
Erp57 thioreductase
TAP-1/TAP-2
Proteolytic enzymes: proteosomes, aminopeptidases (ERAAP/ERAP-1; ERAP-2)

Expression of MHC class I, TAP, LMP-2, -7, MECL-1 and PA28 is induced by IFN-γ

Figure 2B. Viral proteins that block class I assembly



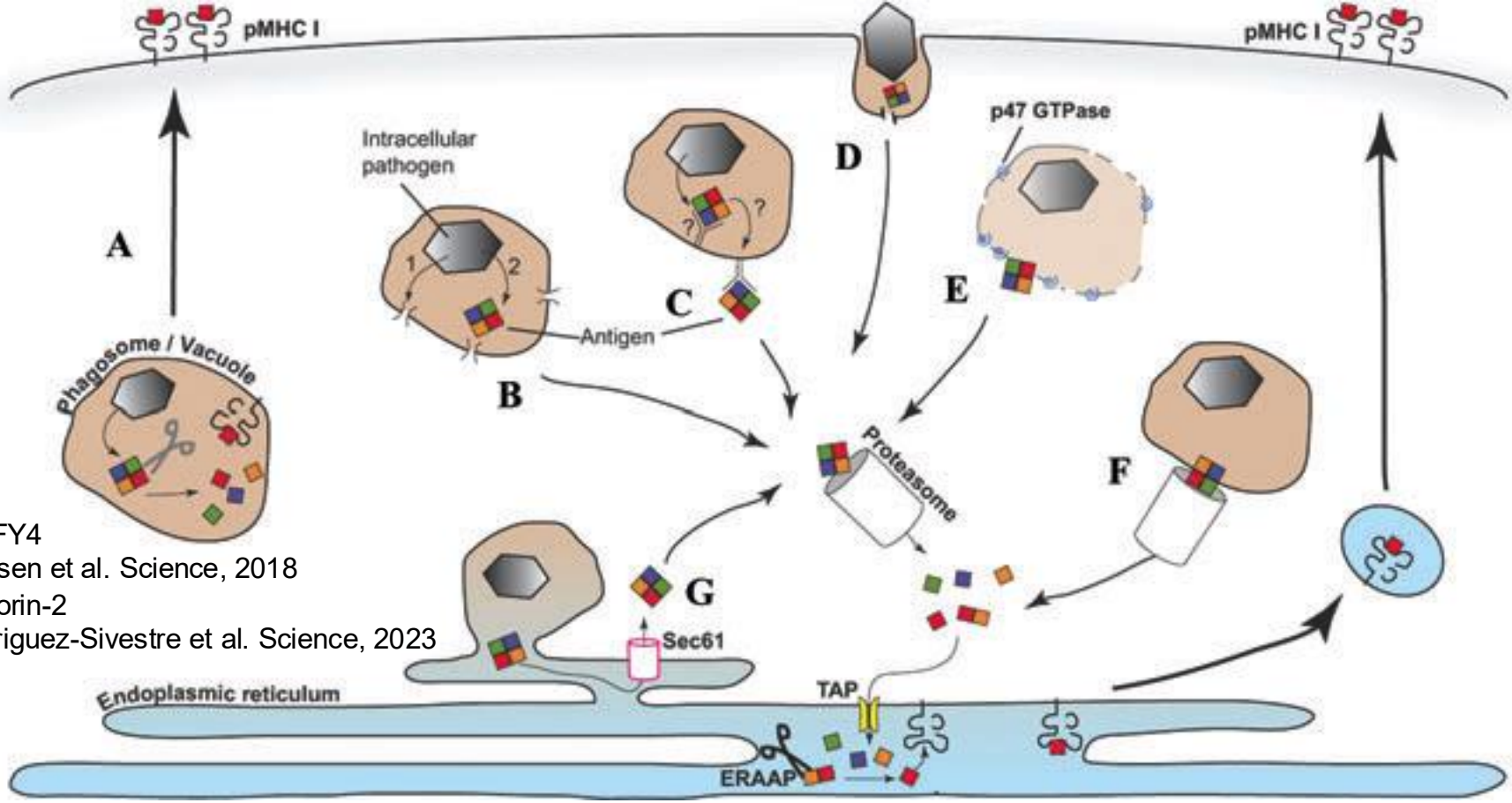
Antigen presenting cells

cTEC
mTEC
Dendritic cells (cDC1; cDC2; PDC, Langerhans cells, etc.)
Macrophages
B cells
Fibroblast-like stromal (FLS) cells

T cells (humans, rats)
Endothelial cells
Epithelial cells

MHC class II regulation by CIITA (RFX5, NF-Y)

CROSS-PRIMING/CROSS-PRESENTATION



WDFY4
Theisen et al. Science, 2018
Perforin-2
Rodriguez-Sivestre et al. Science, 2023

Schematic model for generation of peptide major histocompatibility complex class I (pMHC I) from vacuolar and cytosolic processing pathways. (A) In the transporter associated with antigen processing (TAP)-independent vacuolar pathway, antigens are degraded and loaded on MHC I molecules within the phagosome/vacuole and displayed on the cell surface. (B–G) In the TAP-dependent cytosolic pathway, the antigen gains access to the cytosol where it is processed primarily by the proteasome. Several models have been proposed that differ inthe manner the antigenic material enters the cytoplasm. (B) A secreted factor forms pores in the vacuole membrane, allowing for subsequent diffusion of antigen. (C) The antigen is shuttled to the cytoplasm through an active transport mechanism that may involve recognition of specific motifs and/or a specific translocation machinery. (D) An early break in the plasma membrane during invasion permits passage of the antigen to the cytoplasm ("kiss and spit"). (E) Immunity related GTPases (IRGs) are recruited to the vacuole membrane. Their action mediates membrane disruption and release of potentially antigenic content into the cytosol. (F) The antigen is inserted into the vacuole membrane with a topology consistent with action of the proteasome on the cytosolic side. (G) Direct fusion of the vacuole with the host endoplasmic reticulum (ER) delivers the antigen to the Sec61-based retro-translocation machinery, which shuttles the antigen to the cytoplasm. In all cases, antigen processing occurs in a proteasome-dependent fashion. Antigenic peptide precursors are then imported into the ER for possible trimming by ER aminopeptidase associated with antigen processing (ERAAP) and final MHC I loading.

MHC

(*M*ajor *H*istocompatibility gene *C*omplex)

POLYGENIC and highly POLYMORPHIC gene complex

MHC class II	**Classical MHC class I (Ia)**	**Non-classical MHC class I (Ib)**

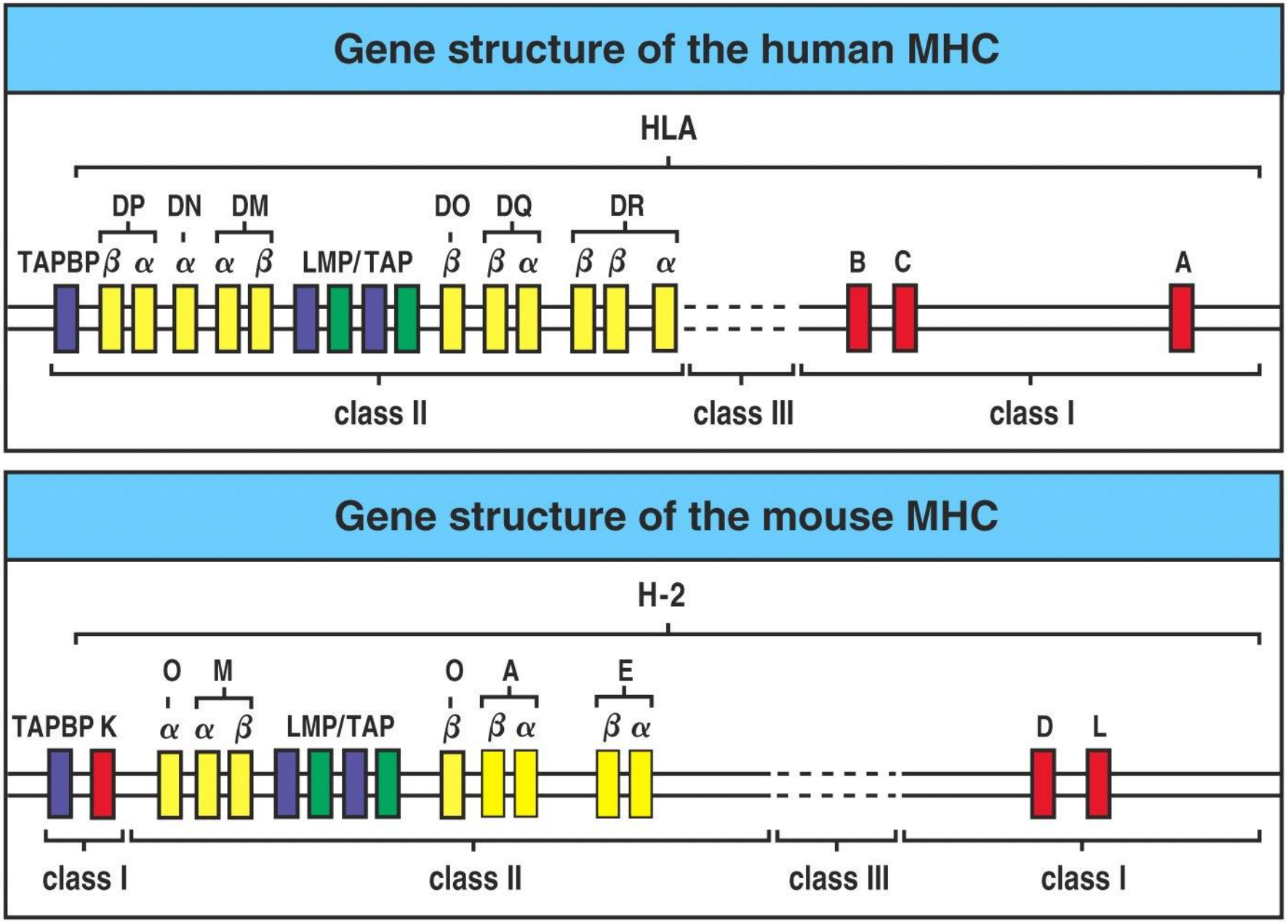
MHC class II	Classical MHC class I (Ia)	Non-classical MHC class I (Ib)
Polymorphic MHC-encoded α and β chains	Polymorphic MHC-encoded α chain+β2m	Non-Polymorphi MHC-encoded α chain+β2m

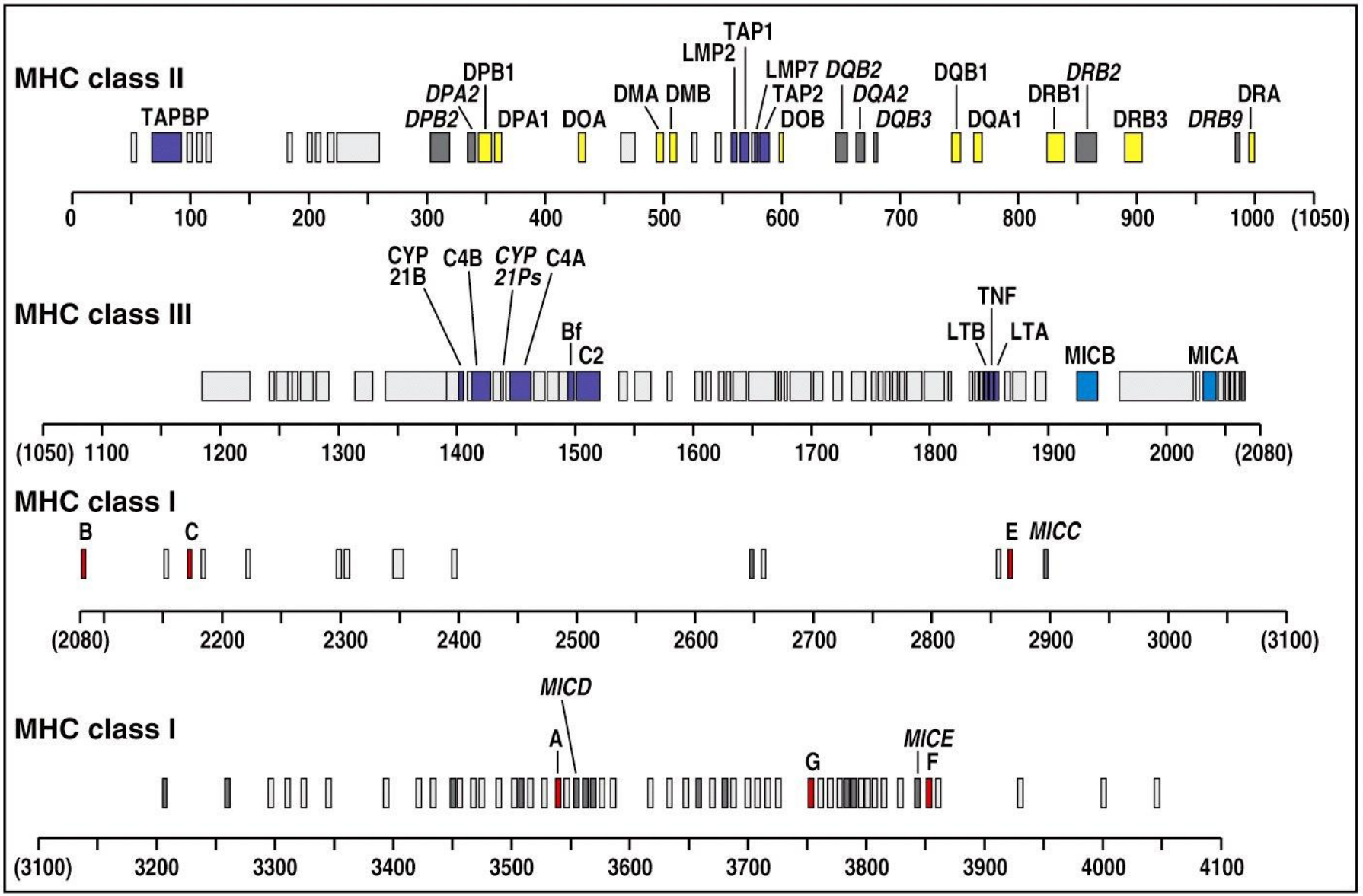
In humans HLA (*H*uman *L*eukocyte *A*ntigen)

HLA-DR	HLA-A	HLA-E, HLA-G
HLA-DQ	HLA-B	MICA/MICB
HLA-DP	HLA-C	

In mice H-2 (*H*istocompatibility locus-*2*)

I-A	H-2K	H-2M3
I-E	H-2D	Qa-1
	H-2L	


Gene structure of the human MHC
HLA
DP DN DM DO DQ DR
TAPBP β α α α β LMP/TAP β β α β β α B C A
class II class III class I
Gene structure of the mouse MHC
H-2
O M O A E
TAPBP K α α β LMP/TAP β β α β α D L
class I class II class III class I


MHC class II
TAPBP
DPB1
DPA2
DPB2
DPA1
DOA
DMA
DMB
TAP1
LMP2
LMP7
TAP2
DOB
DQB2
DQA2
DQB3
DQB1
DQA1
DRB2
DRB1
DRB3
DRB9
DRA
0
100
200
300
400
500
600
700
800
900
1000
(1050)
MHC class III
CYP 21B
C4B
CYP 21Ps
C4A
Bf
C2
TNF
LTB
LTA
MICB
MICA
(1050)
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
(2080)
MHC class I
B
C
E
MICC
(2080)
2200
2300
2400
2500
2600
2700
2800
2900
3000
(3100)
MHC class I
MICD
A
G
MICE
F
(3100)
3200
3300
3400
3500
3600
3700
3800
3900
4000
4100

DISCOVERY OF MHC

Tumor and skin transplantation between INBRED strains of mice

INBRED strains of mice are derived by sequential brother-sister mating. All animals are genetically identical. All alleles are homozygous.

MHC^a -> MHC^a	acceptance
MHC^a -> MHC^b	rejection

MHC POLYMORPHISM

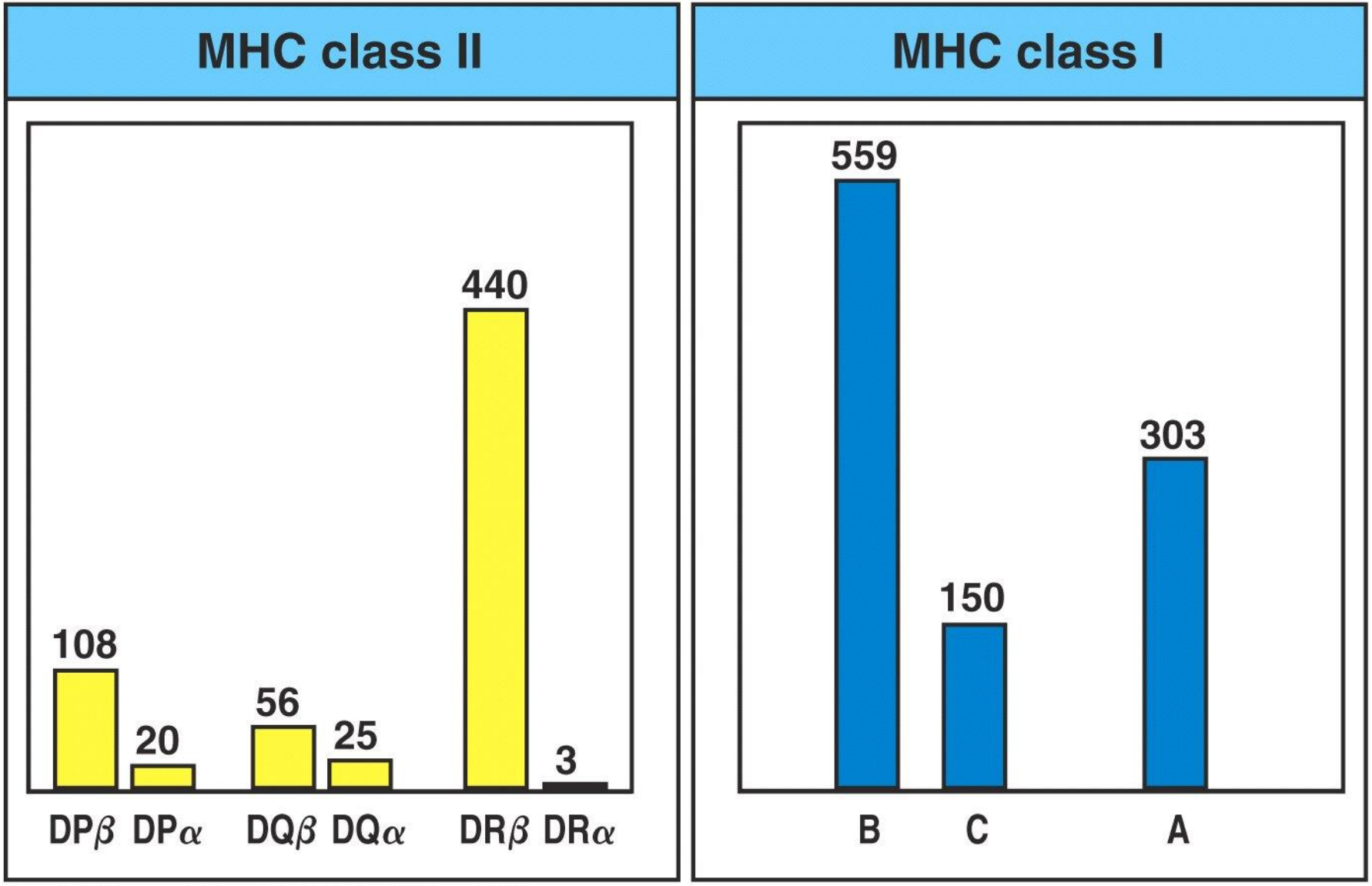
A particular combination of MHC alleles found on a single chromosome is called MHC haplotype

Inbred mice **H-2$^{d, b, k, s\ etc.}$**

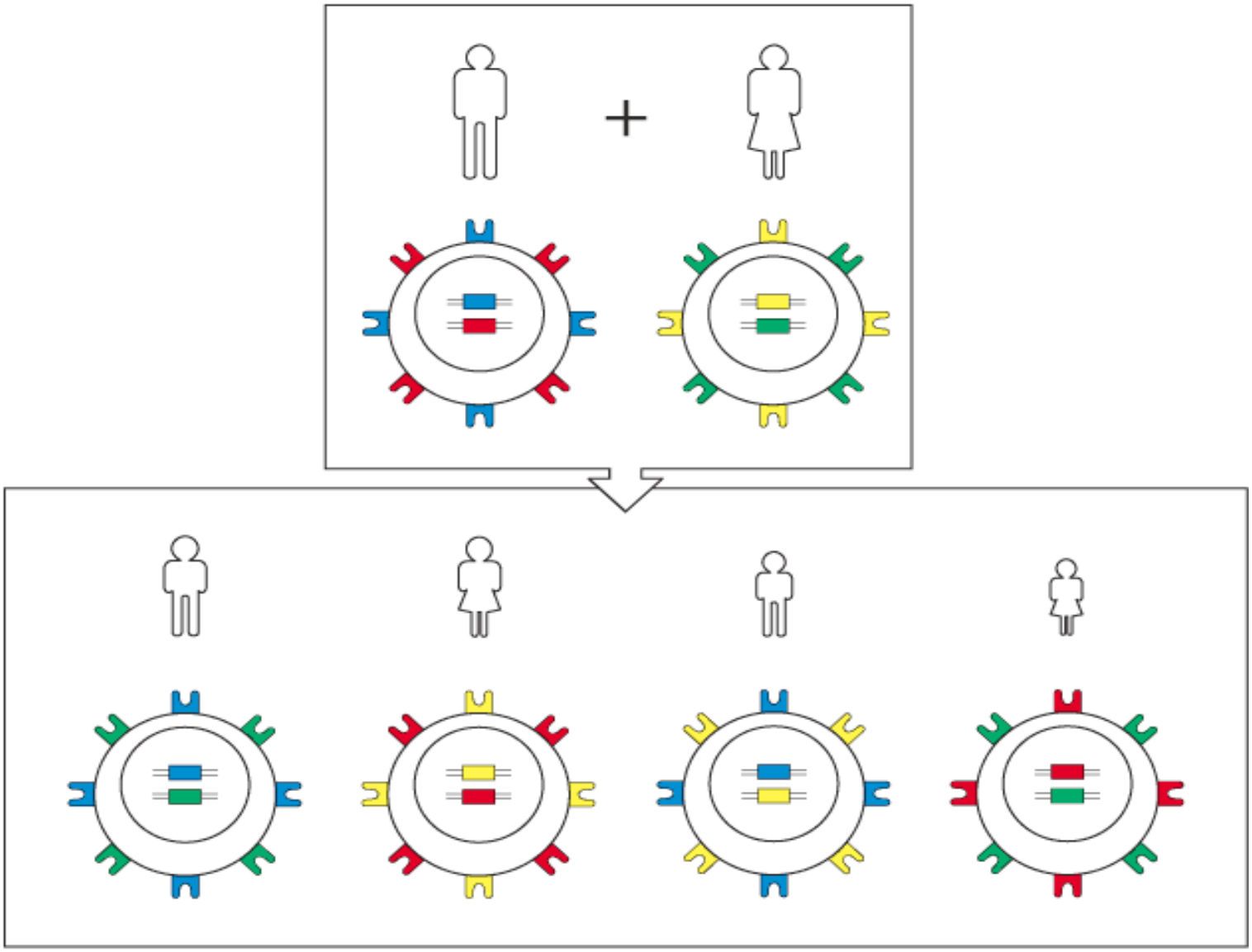
H-2^{d} **H-2K^{d} H-2D^{d} H-2L^{d} I-A^{d} I-E^{d}**

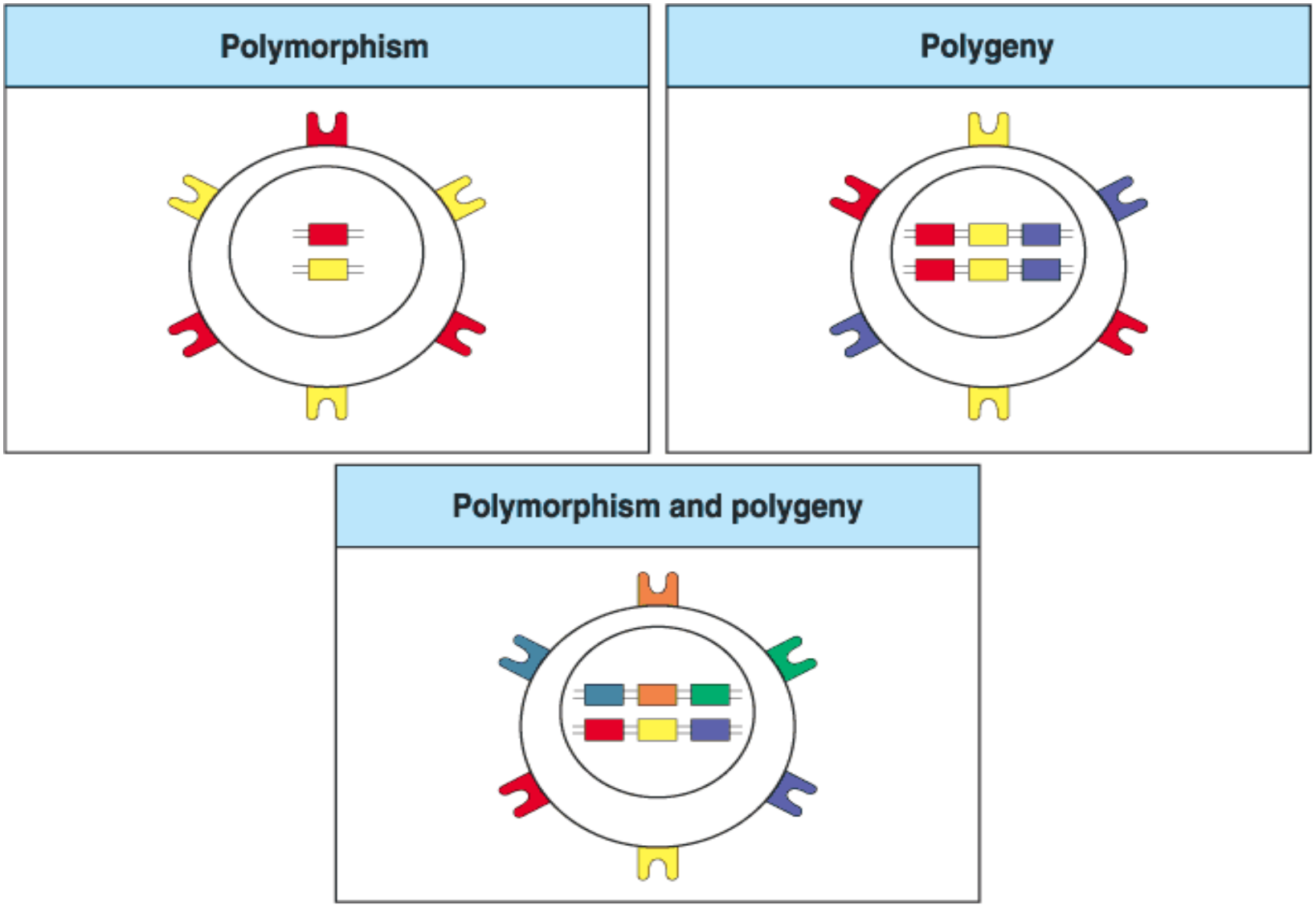
Outbred humans

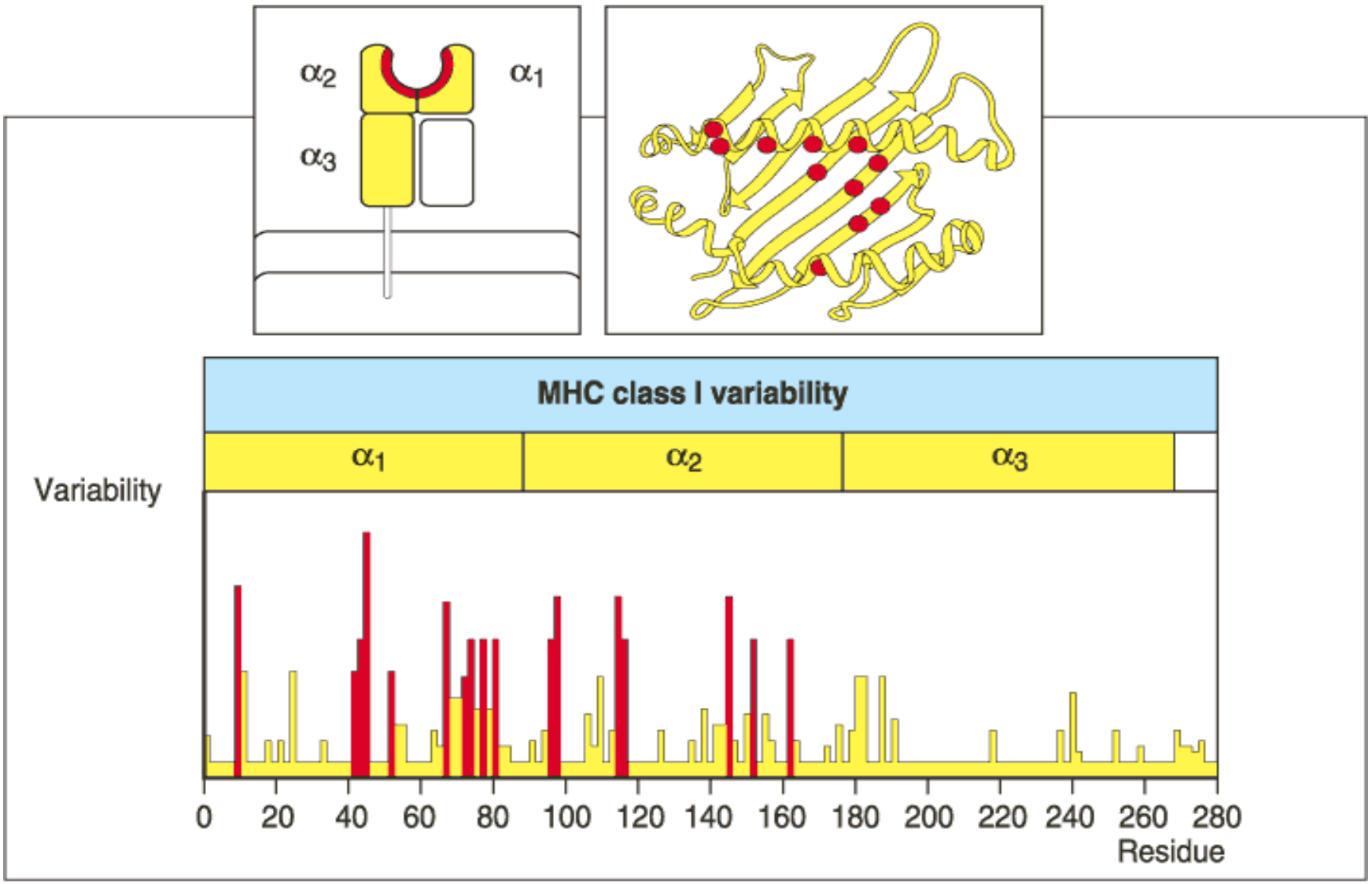
HLA-A2, HLA-B27, HLA-Cw3, HLA-DR4, HLA-DQ52, HLA-DP3

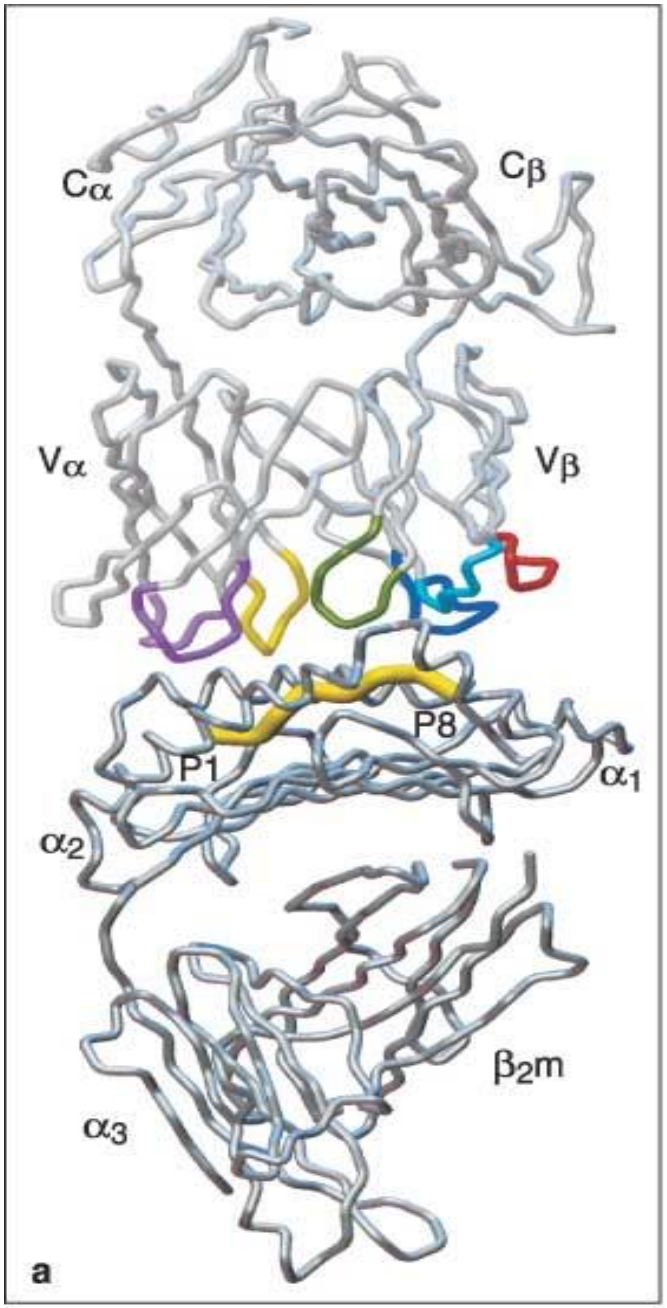

MHC class II
108
20
56
25
440
3
DPβ
DPα
DQβ
DQα
DRβ
DRα
MHC class I
559
150
303
B
C
A

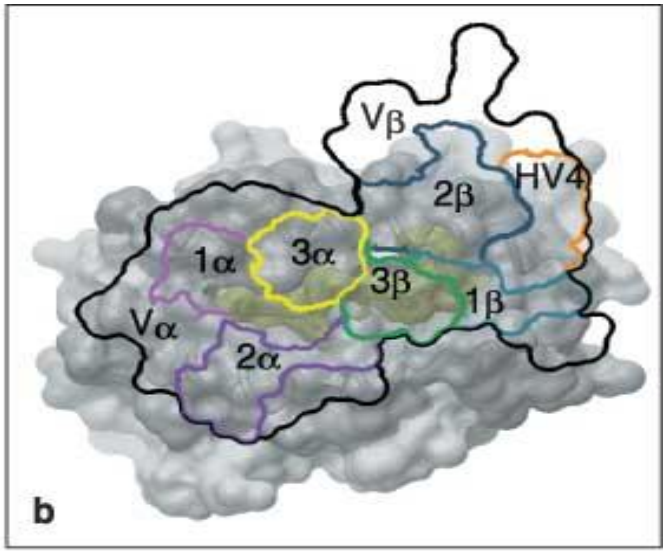
F

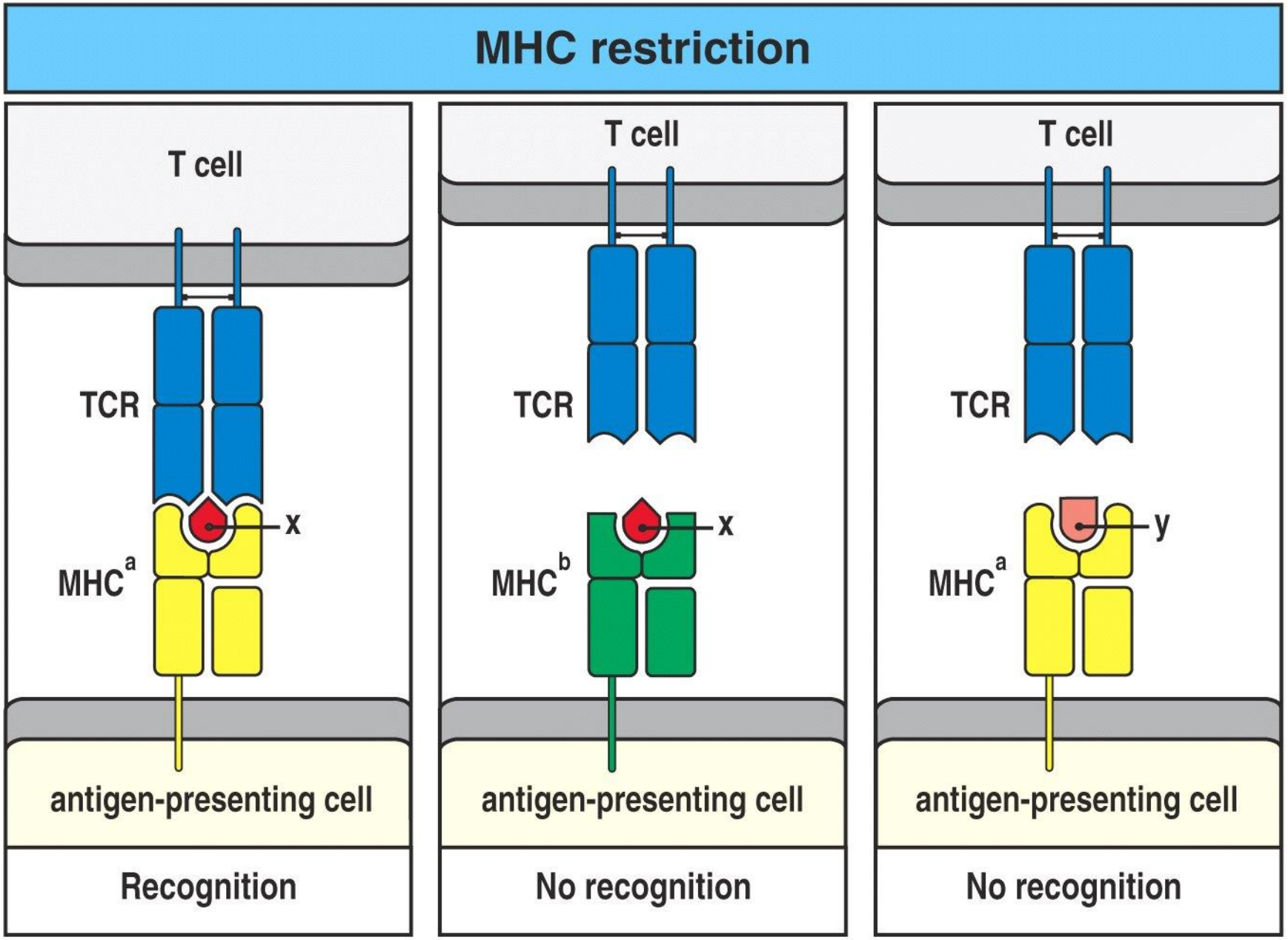



Polymorphism
Polygeny
Polymorphism and polygeny


α2
α1
α3
MHC class I variability
α1
α2
α3
Variability
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
Residue


Cα
Cβ
Vα
Vβ
P8
P1
α1
α2
β2m
α3
a


Vβ
HV4
2β
1α
3α
3β
1β
Vα
2α
b



altered ‘self’ recognition