01

Major Histocompatibility

• Every mammalian species possesses a tightly linked cluster of genes, the major histocompatibility complex (MHC)

•Products play roles in intercellular recognition and in discrimination between self and non-self

• A collection of genes arrayed within a long continuous stretch of DNA on chromosome 6 in humans and on chromosome 17 in mice

• MHC is referred to as the HLA complex in humans and as the H-2 complex in mice

MHC Gene Subtype

1) Class I

2) Class II

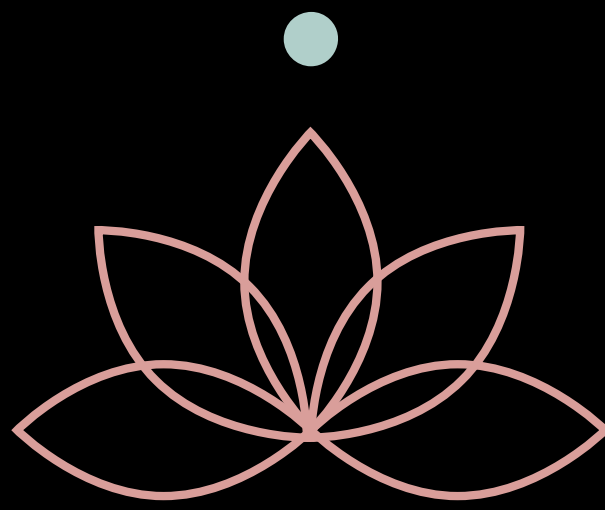
3) Class III

- The loci is highly polymorphic

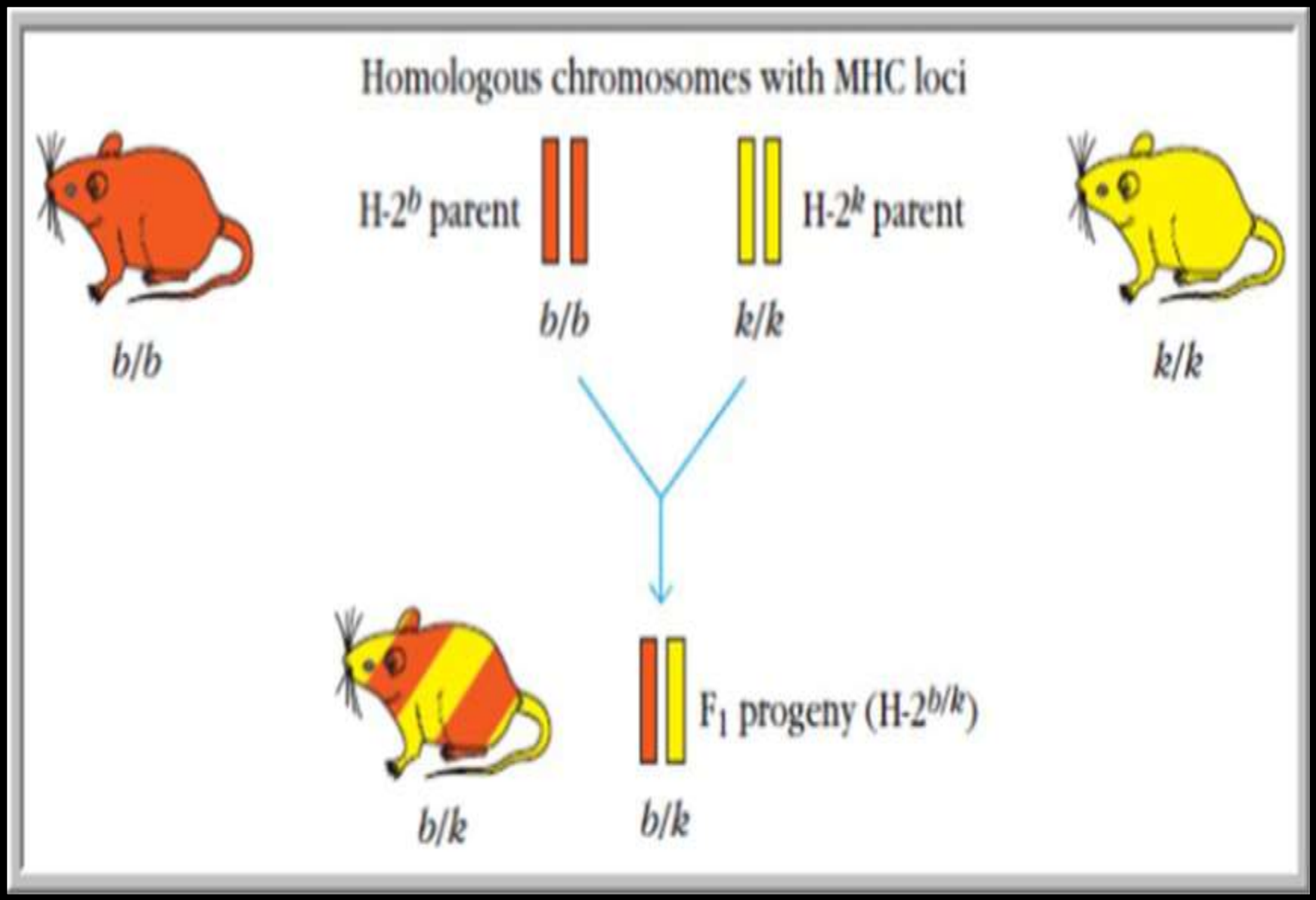
- The MHC loci is closely linked and many alleles exist at each locus.

- Each sets of alleles referred to as "haplotype".

- The alleles are co dominantly expressed.



Inheritance of MHC haplotypes in inbred mouse strains



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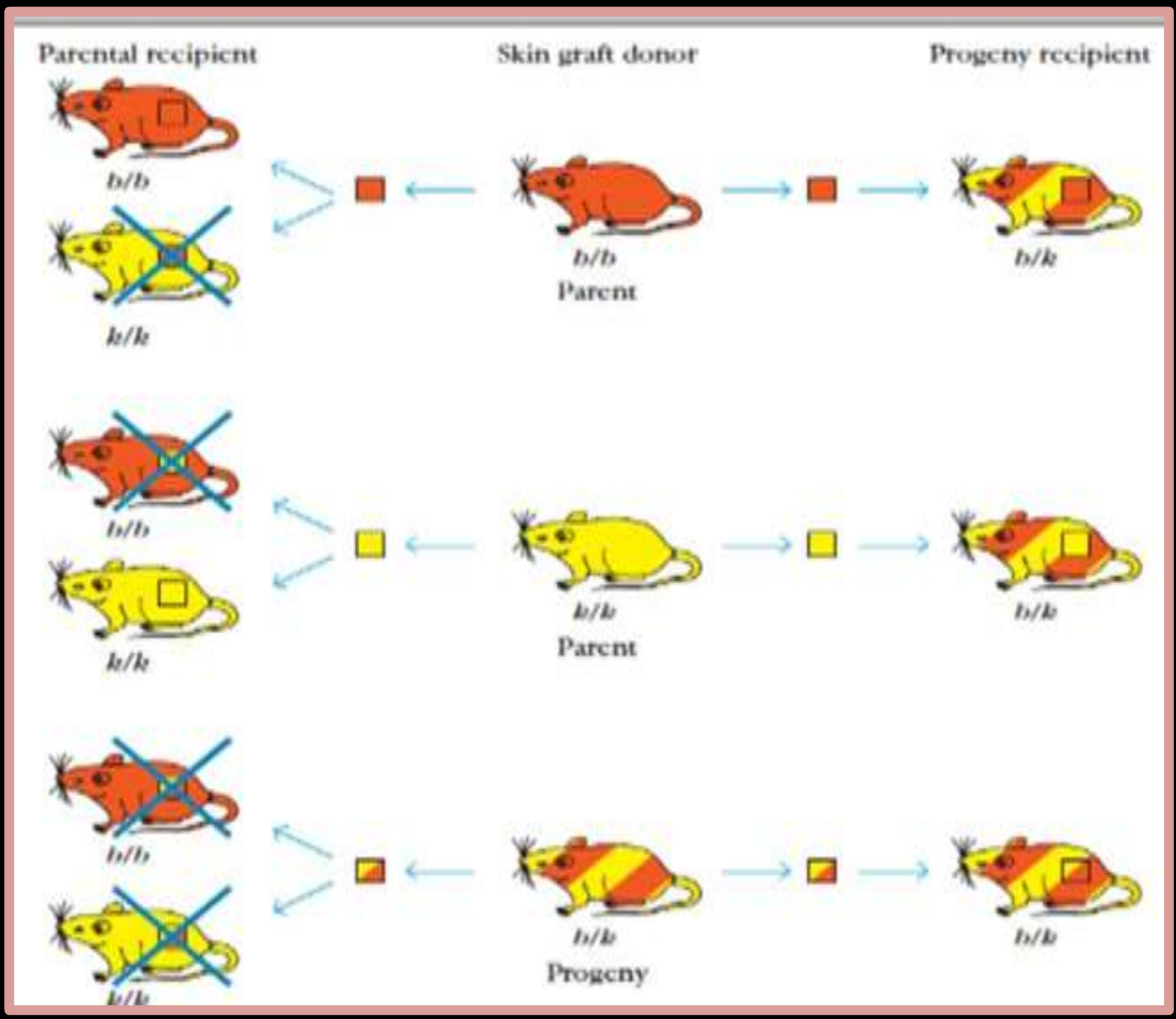


MHC Molecule

- Class I and class II MHC molecules are membrane-bound glycoproteins
- Are closely related in both structure and function
- Function as highly specialized antigen-presenting molecules that form unusually stable complexes with antigenic peptides, displaying them on the cell surface for recognition by T cells
- Class III MHC molecules are a group of unrelated proteins that do not share structural similarity and common function with class I and II molecules

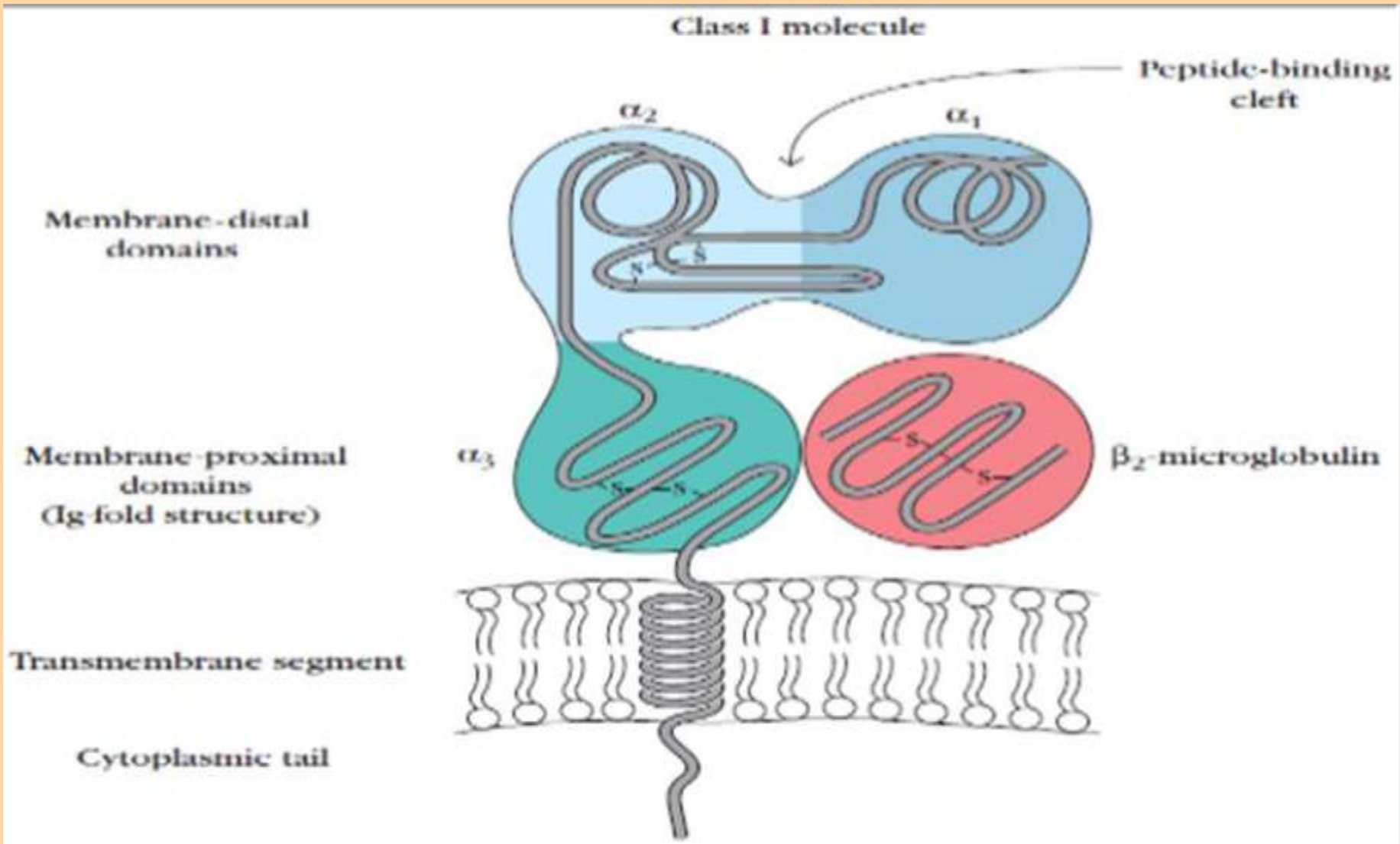
Structure of Class I MHC

- Class I MHC molecules contain a 45-kilodalton (kDa) a chain associated non-covalently with a 12-kDa B2-microglobulin molecule

- alpha-subunit is a glycoprotein encoded by A,B And C regions of HLA complex

- B_2-Micro globulin is a protein encoded by a highly conserved gene located on a different chromosome

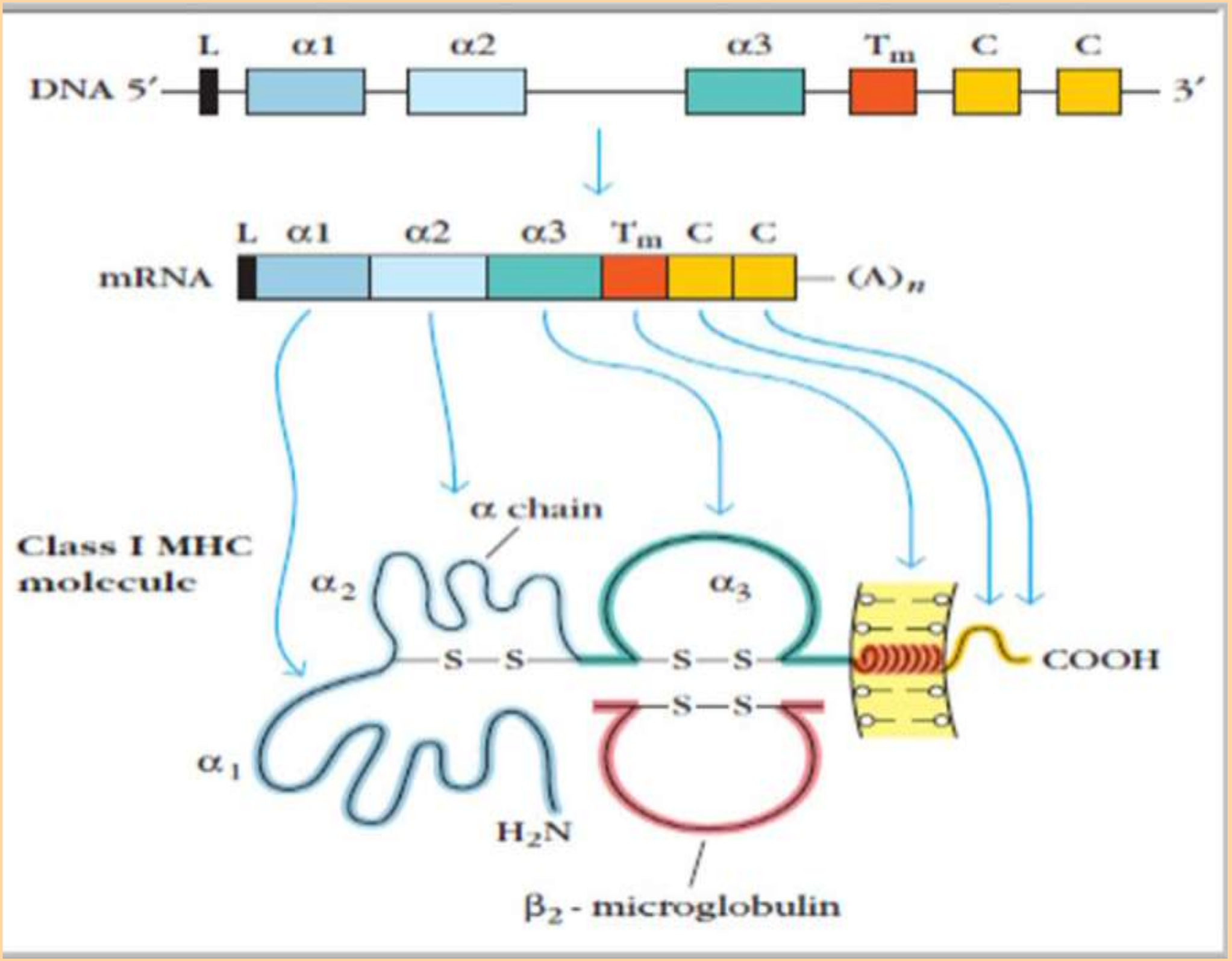
- alpha chain is organized into three external domains (a1,02,03) each approximately of 90 amino acids, a transmembrane domain of about 25 hydrophobic amino acids followed by short stretch of charged (hydrophilic) amino acids; and a cytoplasmic anchor segment of 30 amino acids



- Peptide binding cleft formed by al and a2 domains where 8 10 amino acid can bind

- α3-ß2-microglobulin resembles Ig fold

Arrangement of exons in MHC gene



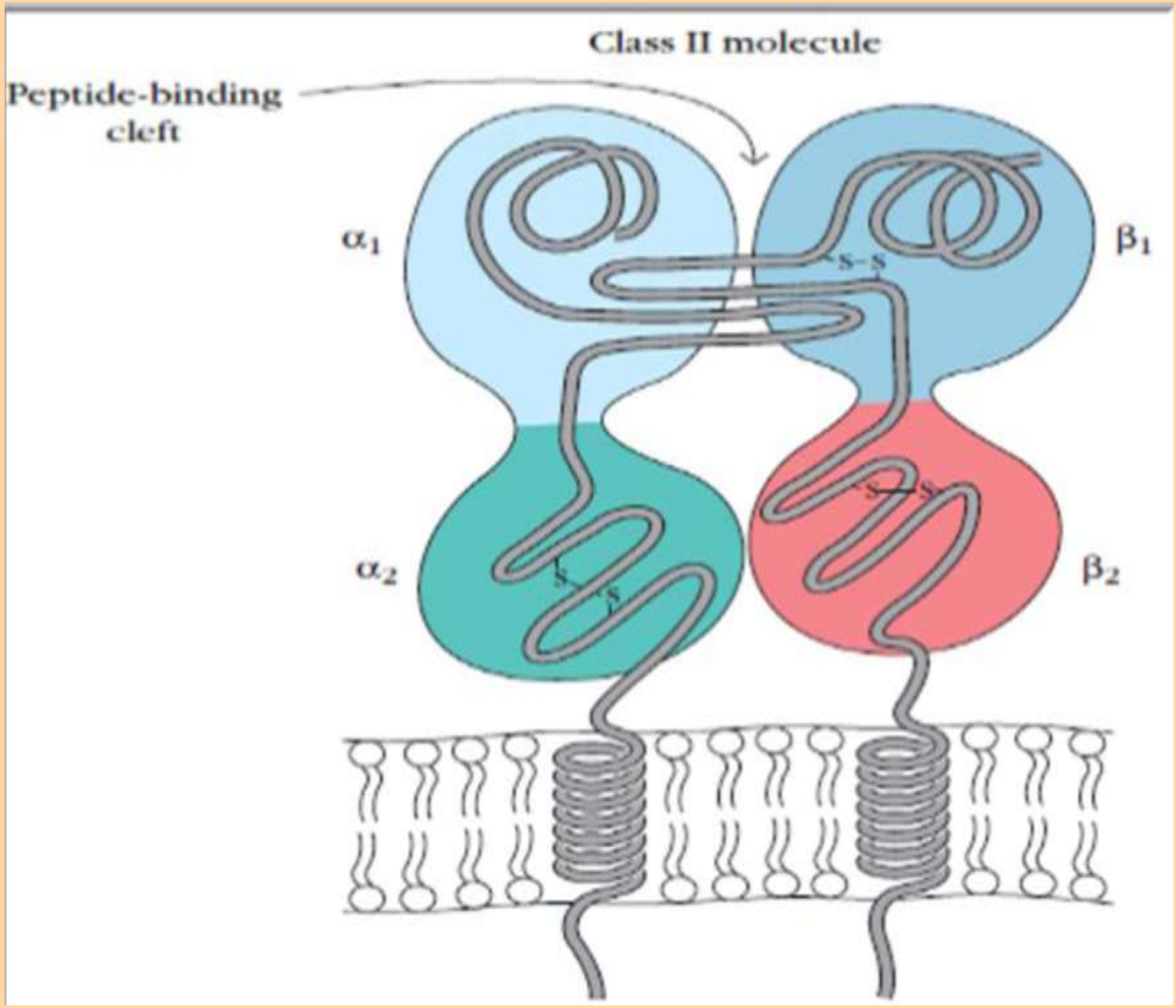
Structure of class II MHC

- Class II MHC molecules contain two different polypeptide chains (membrane bound glycoprotein), a 33-kDa a chain and a 28-kDa ß chain, which associate by non covalent interactions

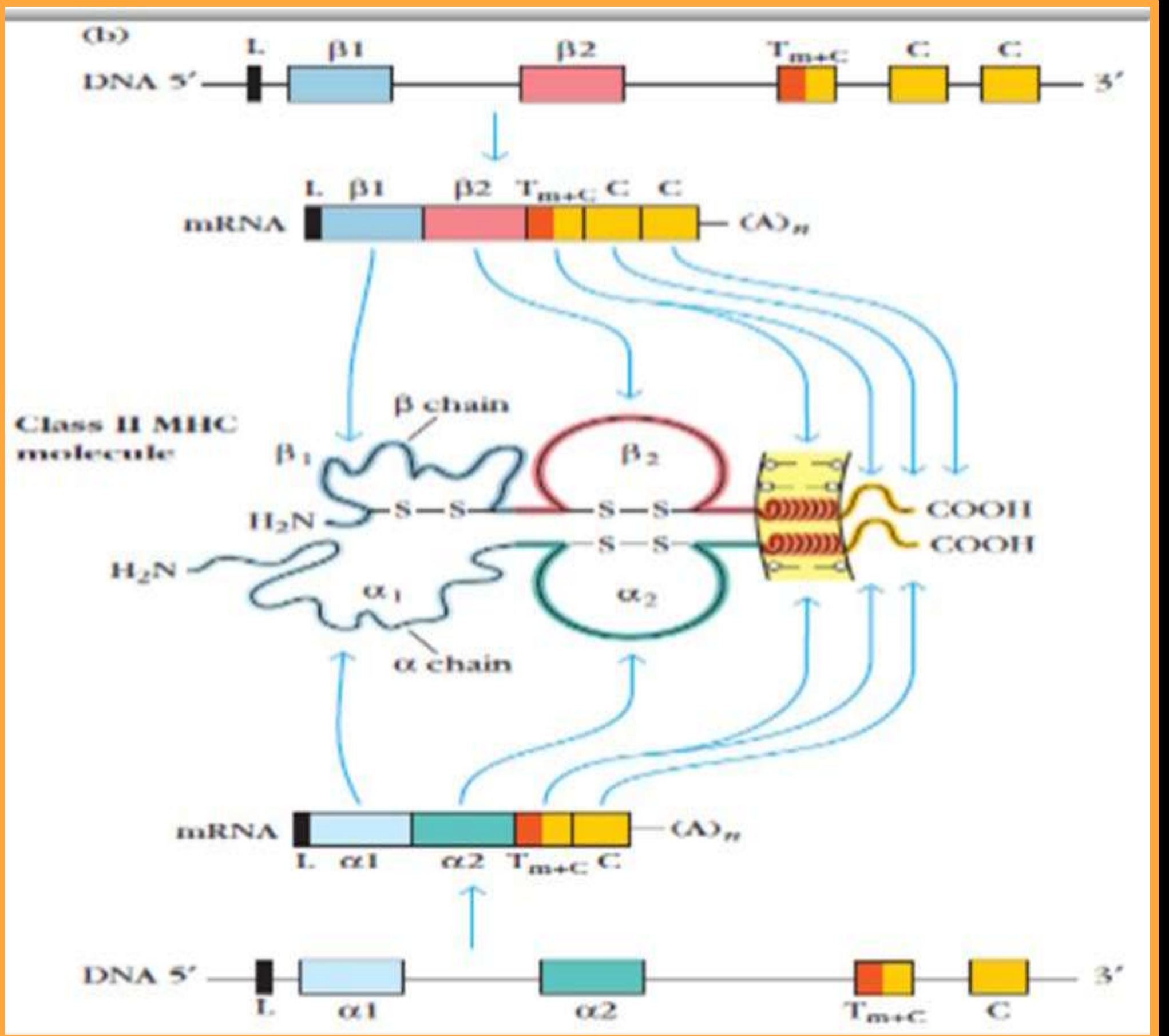
- Contain external domains, a transmembrane segment, and a cytoplasmic anchor segment

- Each chain contains two external domains: αl and α2 domains in one chain and ß1 and ß2 domains in the other

- αl and ß1 domains and forms the antigen-binding cleft for processed antigen.a2 and 32 domains resembles Ig fold


Class II molecule
Peptide-binding cleft
α1
β1
α2
β2
S–S
S–S
S–S

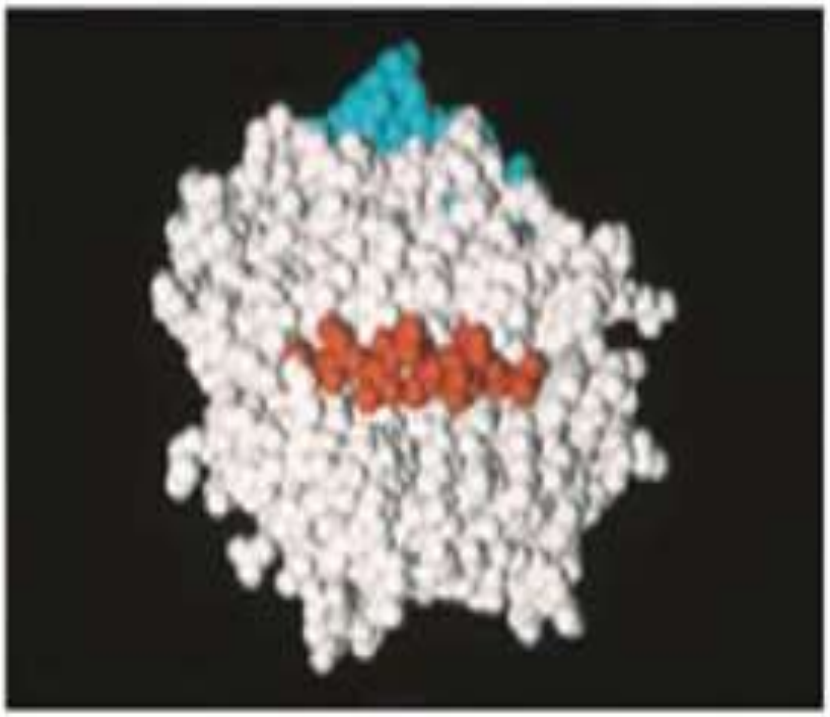
Arrangement of exons in MHC gene



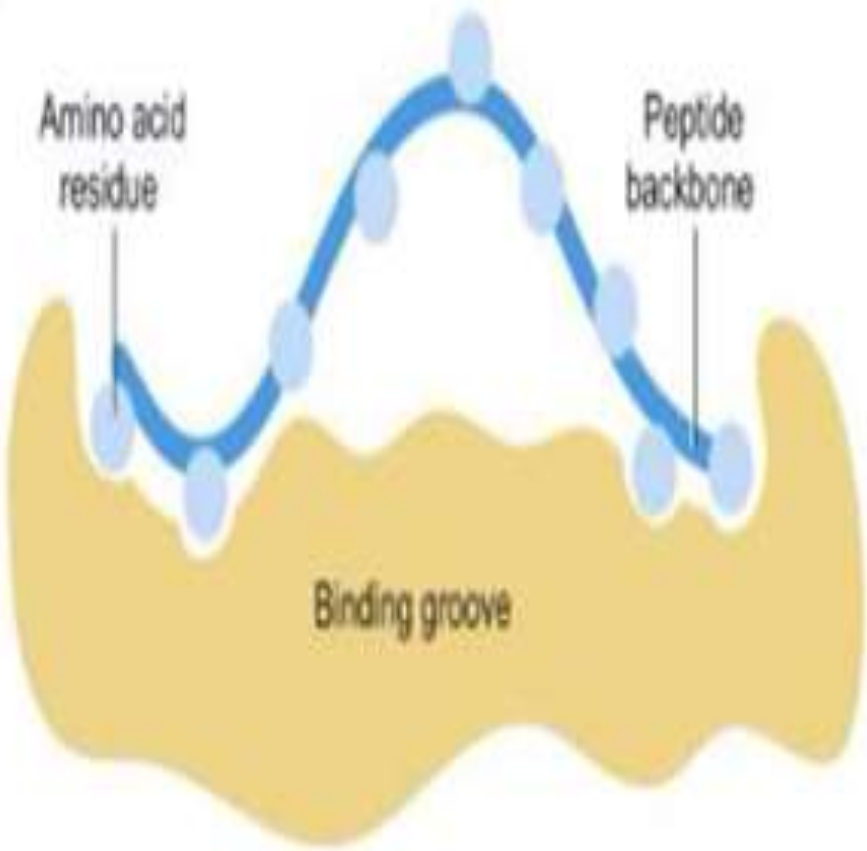
Peptide Binding By Class I and class II

	Class I molecules	Class II molecules
Peptide-binding domain	α1/α2	α1/β1
Nature of peptide-binding cleft	Closed at both ends	Open at both ends
General size of bound peptides	8–10 amino acids	13–18 amino acids
Peptide motifs involved in binding to MHC molecule	Anchor residues at both ends of peptide; generally hydrophobic carboxyl-terminal anchor	Anchor residues distributed along the length of the peptide
Nature of bound peptide	Extended structure in which both ends interact with MHC cleft but middle arches up away from MHC molecule	Extended structure that is held at a constant elevation above the floor of MHC cleft

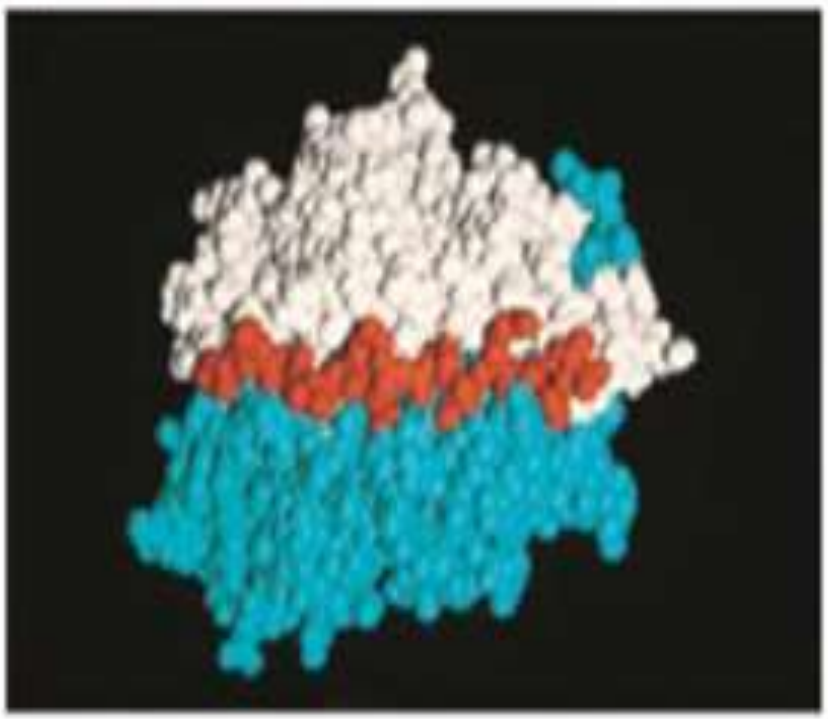
(a) Class I MHC



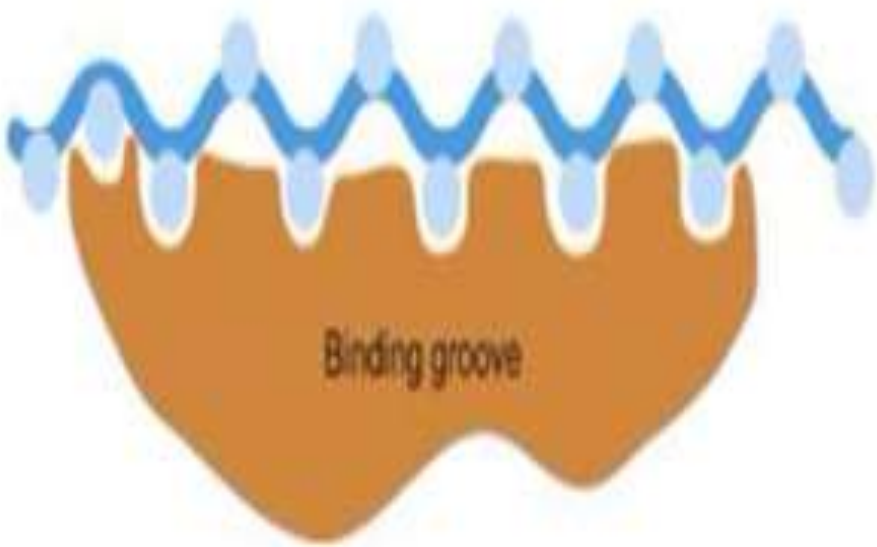
(A) Peptide in MHC Class I Binding Groove



(b) Class II MHC



(B) Peptide in MHC Class II Binding Groove



Regulation of MHC Expression

- Transcriptional regulation of the MHC is mediated by both positive and negative elements - MHC II trans activator, called CIITA, and transcription factor, called RFX.

- Cytokines - interferons (alpha, beta, and gamma) and tumor necrosis factor have each been shown to increase expression of class I MHC molecules on cells

- IFN-y also has been shown to induce expression of the class II transactivator (CIITA), thereby indirectly increasing expression of class II MHC molecules on a variety of cells, including non-antigen-presenting cells

- IL-4 increases expression of class II molecules by resting B cells where as IFN-y, corticosteroids and prostaglandins decrease the expression

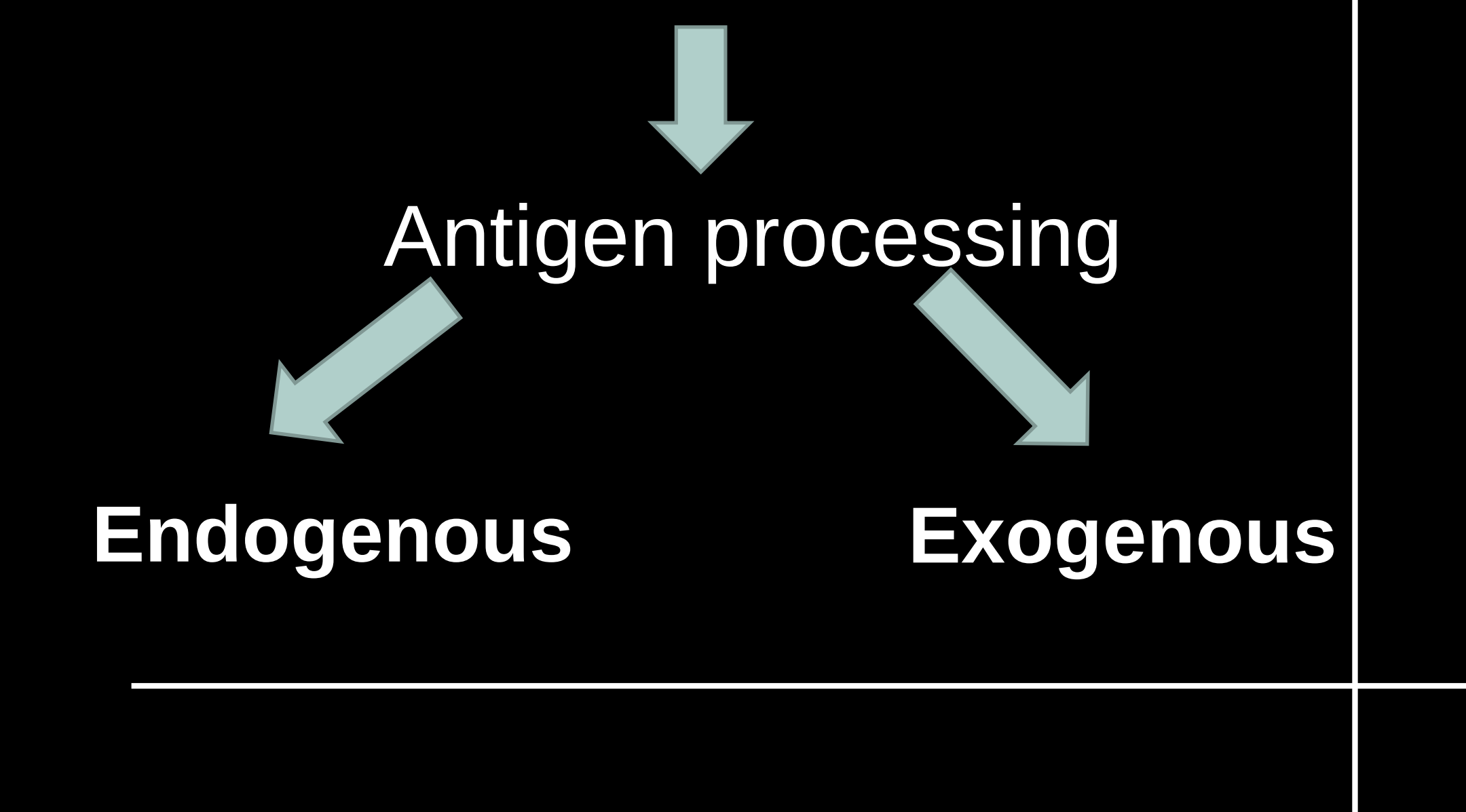
- MHC expression is decreased by infection with certain viruses, including human cytomegalovirus (CMV), hepatitis B virus (HBV),and adenovirus 12 (Ad12)

02

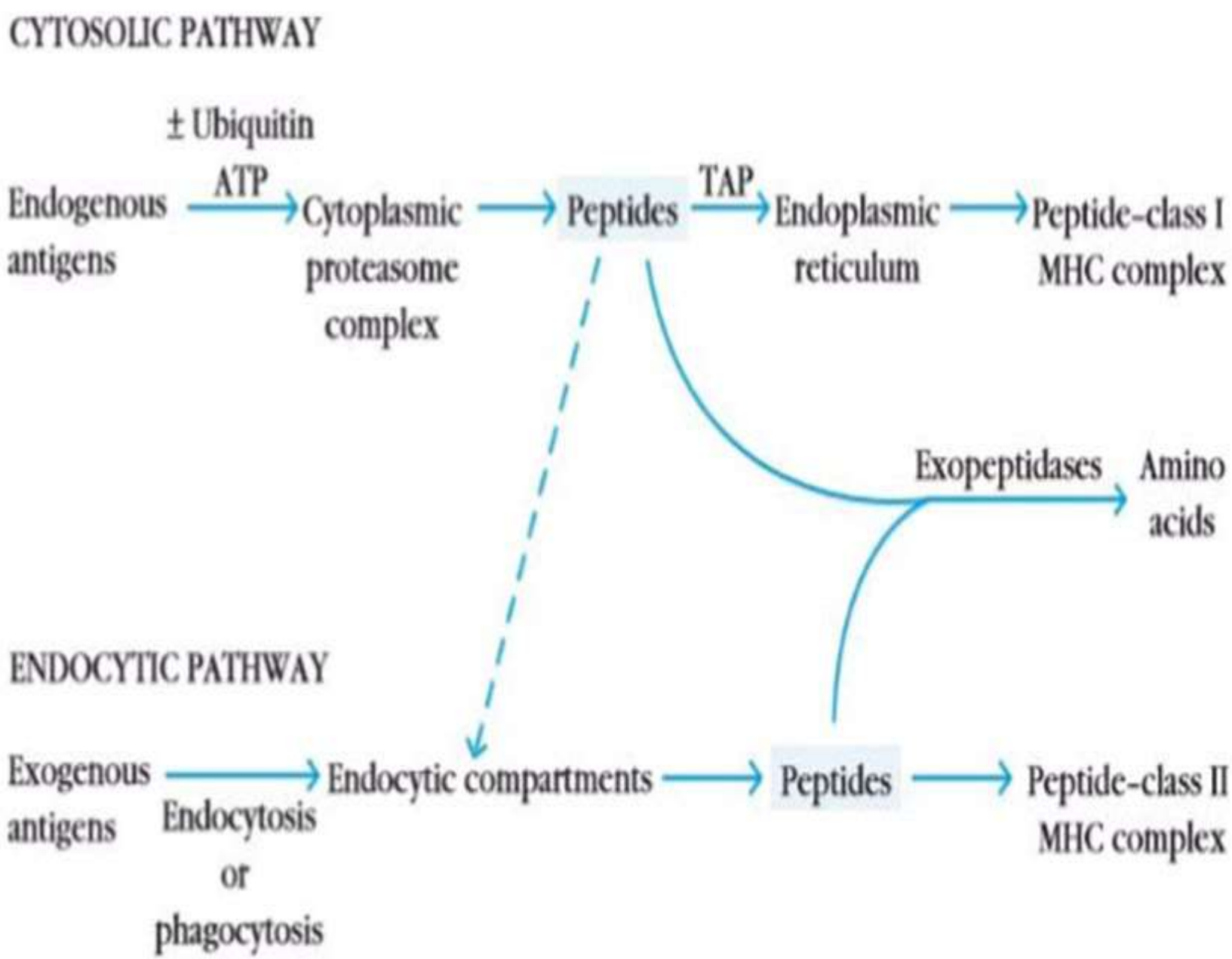
Antigen Processing and Presentation

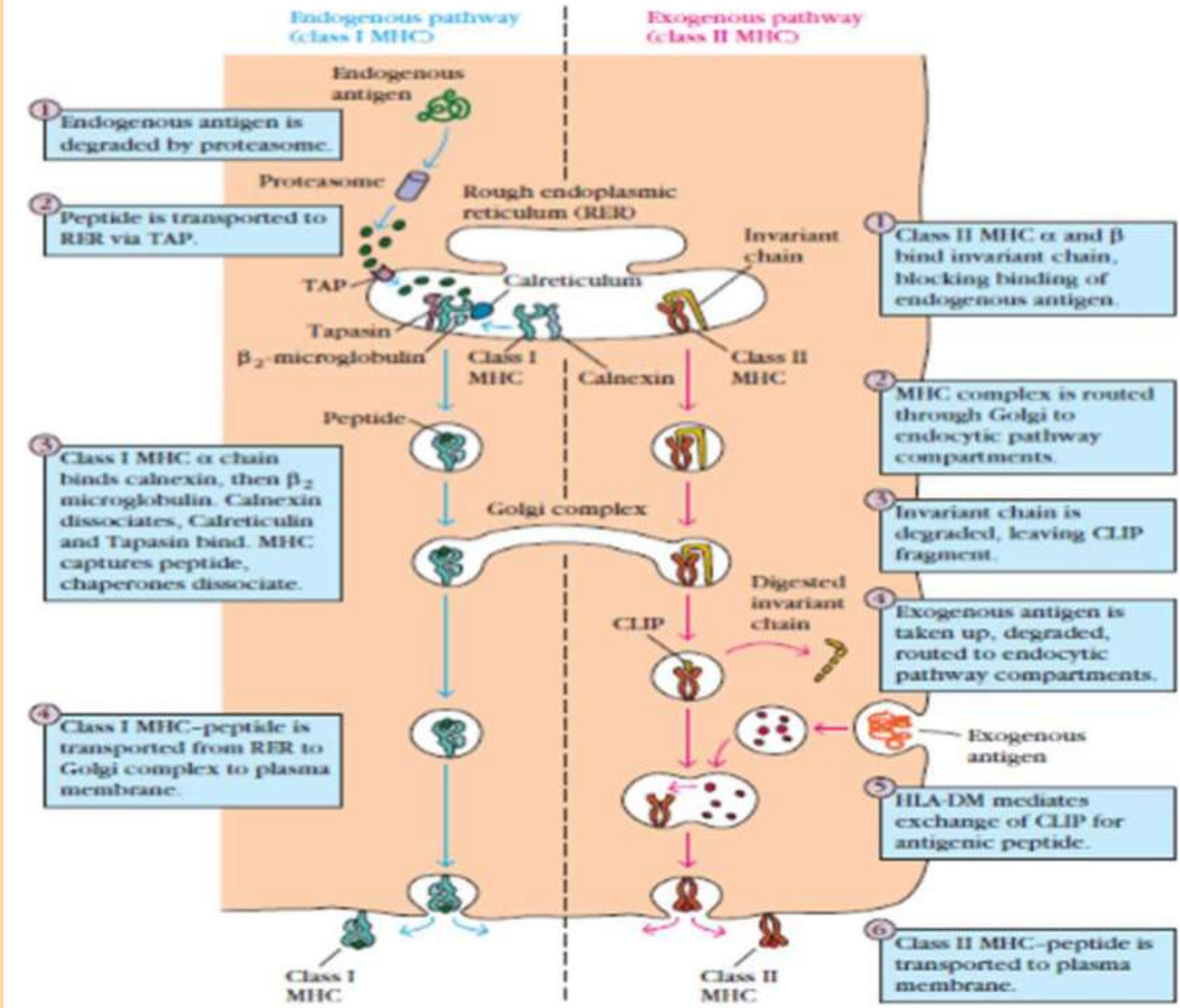
- T cells require antigenic peptides to be displayed by MHC on APC

- Antigenic protein degraded into peptides



EXOGENOUS	ENDOGENOUS
• Exogenous antigens (phagocytosis)	• Endogenous antigens processed within cell
• Processing done in the endocytic pathway	• Processing done in the cytosolic pathway
• Presented on the membrane with class II MHC molecules Pathogen in APC	• Presented on the membrane with class I MHC molecules
	• Transcription factors. cyclins. denatured proteins. viral protein


CYTOSOLIC PATHWAY
± Ubiquitin
ATP
TAP
Endogenous antigens
Cytoplasmic proteasome complex
Peptides
Endoplasmic reticulum
Peptide-class I MHC complex
Exopeptidases
Amino acids
ENDOCYTIC PATHWAY
Exogenous antigens
Endocytosis or phagocytosis
Endocytic compartments
Peptides
Peptide-class II MHC complex


Endogenous pathway (class I MHC)
Exogenous pathway (class II MHC)
Endogenous antigen
1 Endogenous antigen is degraded by proteasome.
Proteasome
2 Peptide is transported to RER via TAP.
Rough endoplasmic reticulum (RER)
Invariant chain
TAP
Calreticulum
Tapasin
β_2-microglobulin
Class I MHC
Calnexin
Class II MHC
1 Class II MHC α and β bind invariant chain, blocking binding of endogenous antigen.
2 MHC complex is routed through Golgi to endocytic pathway compartments.
Peptide
3 Class I MHC α chain binds calnexin, then β_2 microglobulin. Calnexin dissociates, Calreticulin and Tapasin bind. MHC captures peptide, chaperones dissociate.
Golgi complex
Digested invariant chain
CLIP
3 Invariant chain is degraded, leaving CLIP fragment.
4 Exogenous antigen is taken up, degraded, routed to endocytic pathway compartments.
4 Class I MHC–peptide is transported from RER to Golgi complex to plasma membrane.
Exogenous antigen
5 HLA-DM mediates exchange of CLIP for antigenic peptide.
Class I MHC
Class II MHC
6 Class II MHC–peptide is transported to plasma membrane.

Cytosolic Pathway

- **Peptide Generation**

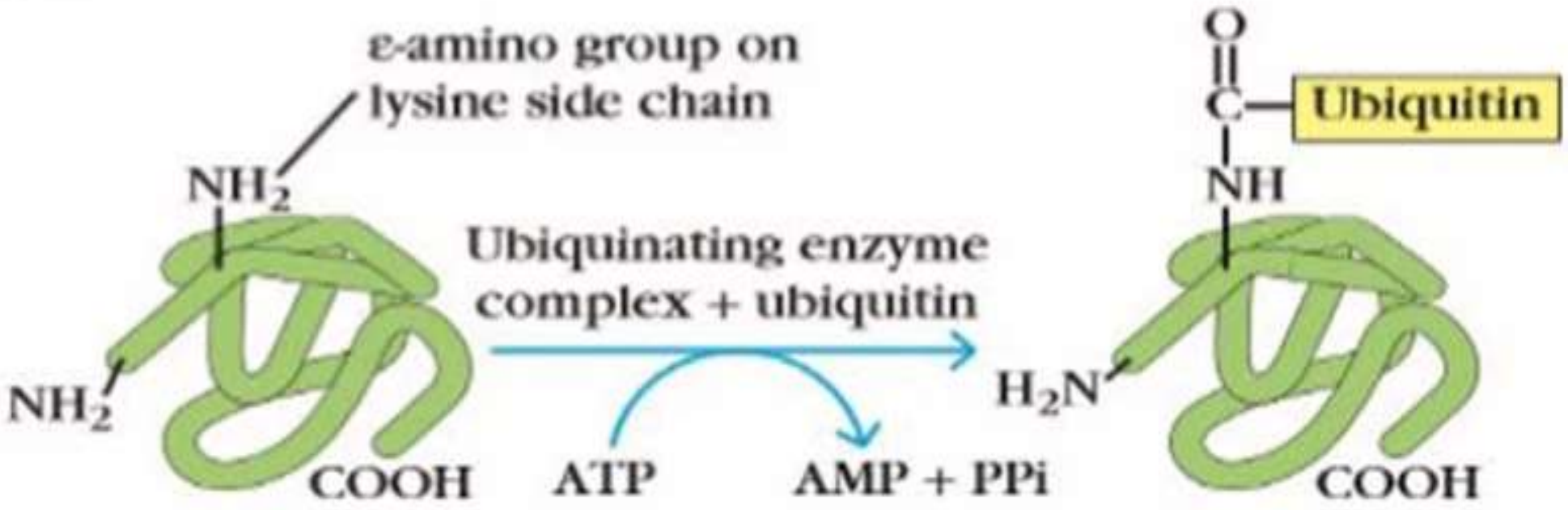
- Intracellular proteins are degraded into short peptides by a cytosolic proteolytic system present in all cells

- Proteins targeted for proteolysis often have a small protein, called ubiquitin, attached to them

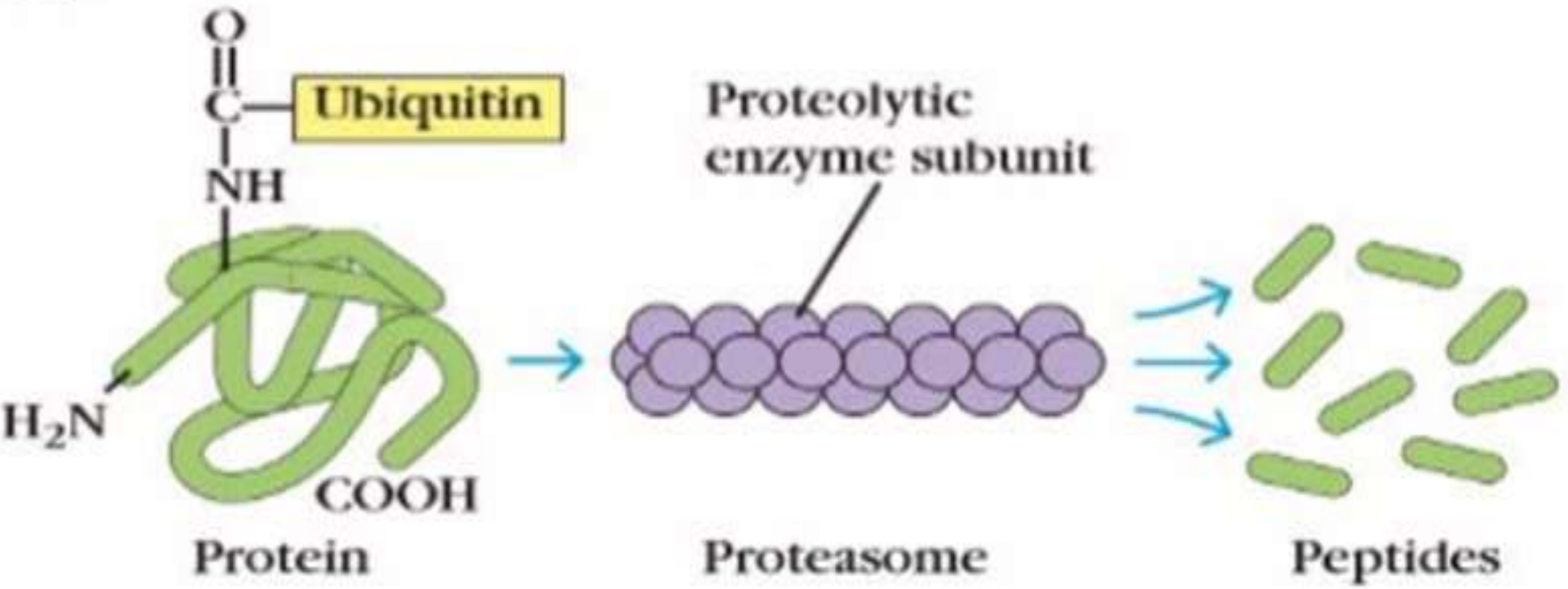
- Ubiquitin-protein conjugates can be degraded by a multifunctional protease complex called a proteasome

- A proteasome can cleave peptide bonds between 2 or 3 different amino acid combinations in an ATP-dependent process

(a)


ε-amino group on
lysine side chain
NH2
NH2
COOH
Ubiquinating enzyme
complex + ubiquitin
ATP
AMP + PPi
O
C
Ubiquitin
NH
H2N
COOH

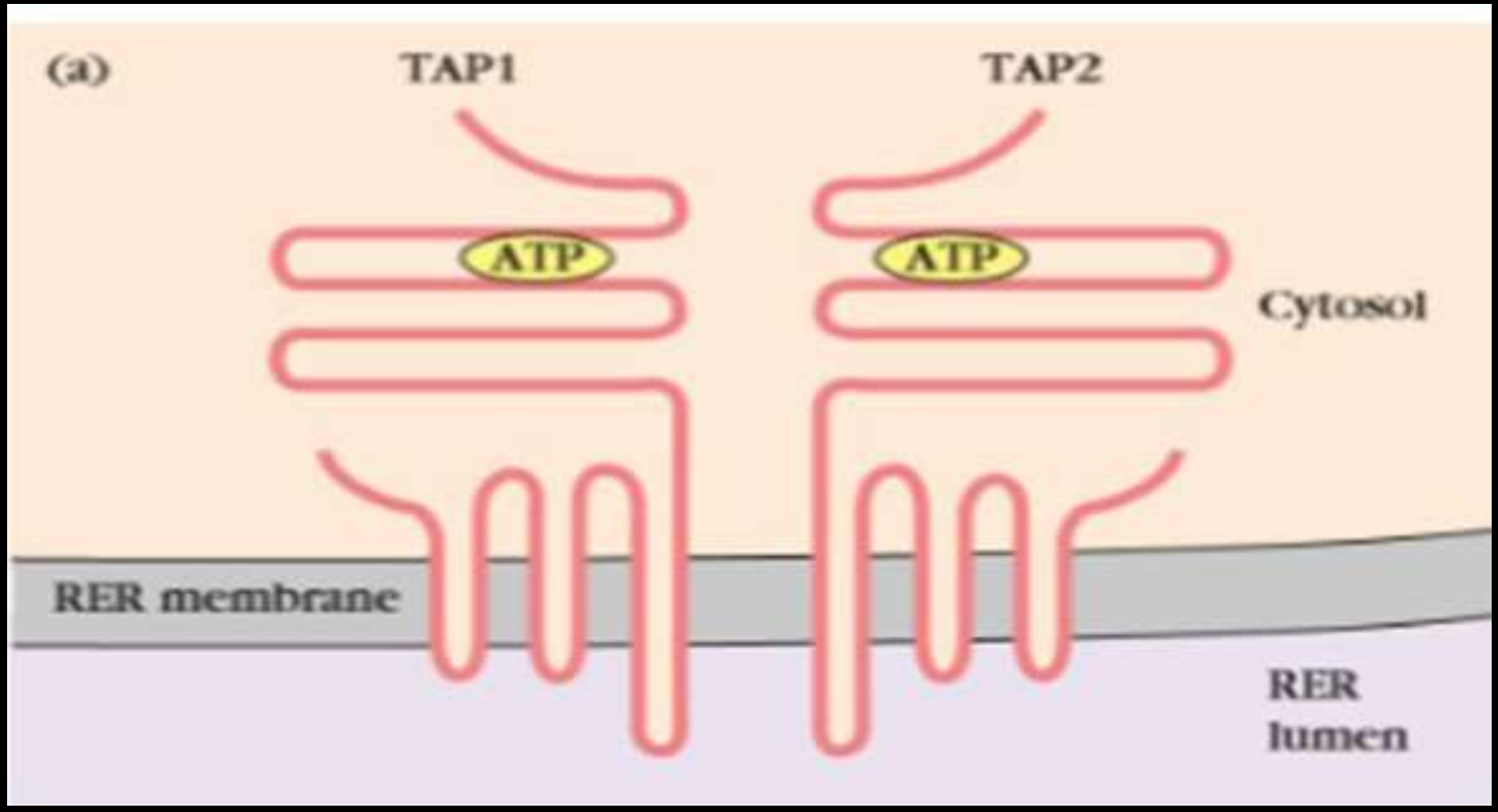
(b)


O
C
Ubiquitin
NH
H2N
COOH
Protein
Proteolytic
enzyme subunit
Proteasome
Peptides

Peptide Transport to RER

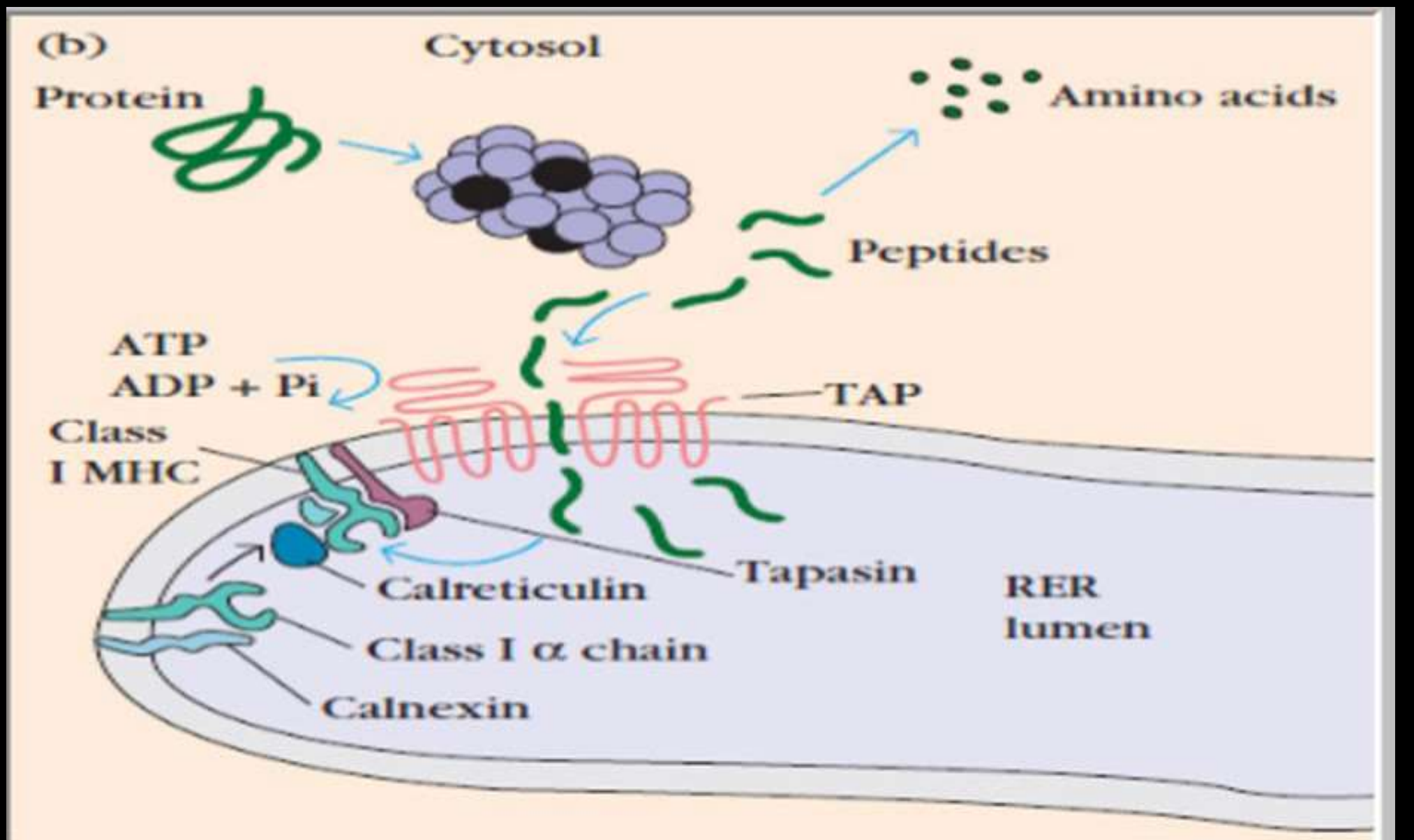
- Peptides generated in the cytosol by the proteasome are trans located by TAP (transporter associated with antigen processing) into the RER by a process that requires the hydrolysis of ATP

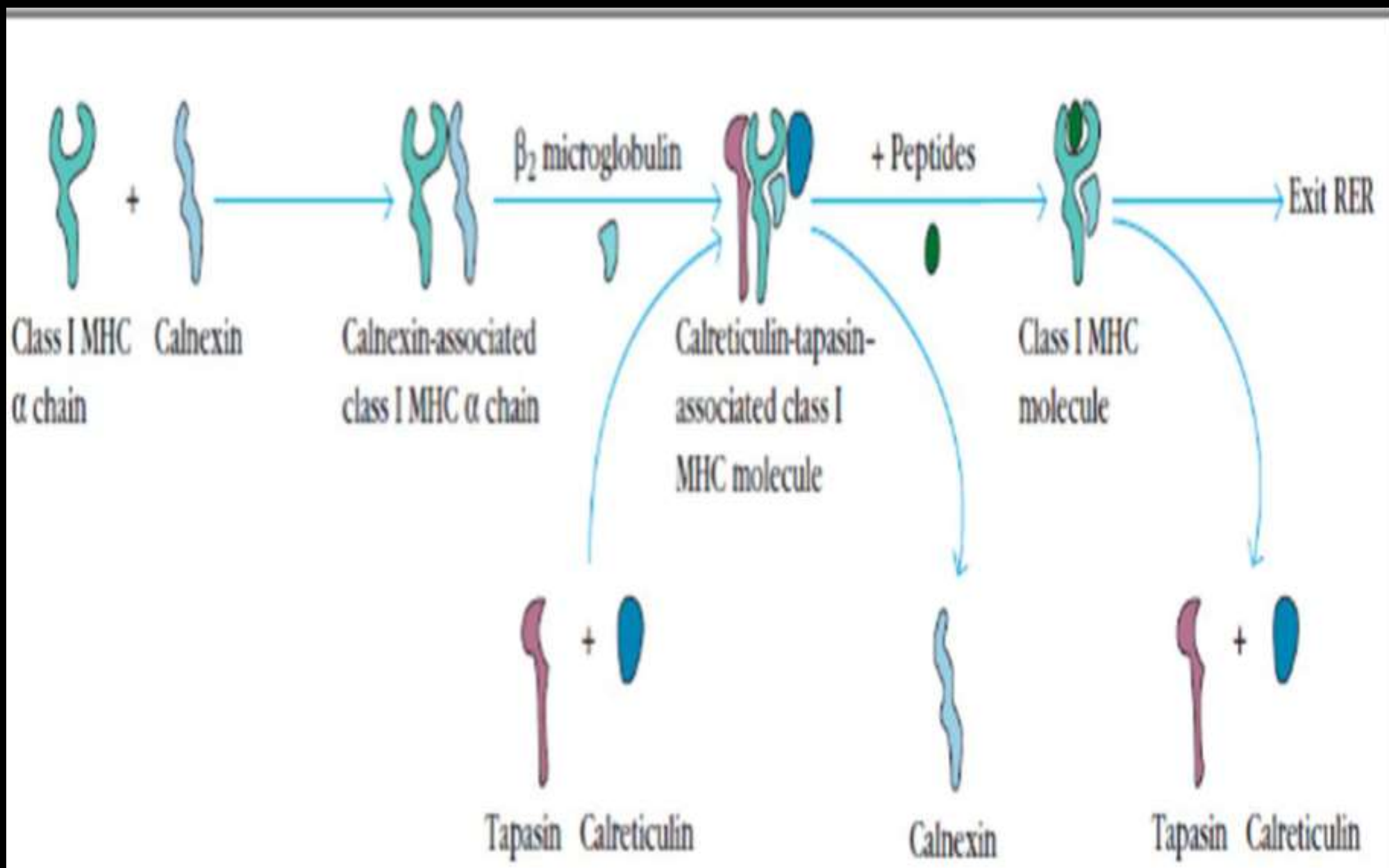
  TAP has the highest affinity for peptides containing 8-10 amino acids with hydrophobic or basic carboxyl-terminal amino acids



Peptide assembly with Class I MHC

- ✓ Assembly of stable class I MHC molecular complex that can exit the RER requires the presence of a peptide in the binding groove of the class I molecule
- ✓ Calnexin associates with the free class I a chain and promotes its folding
- ✓ When B2-microglobulin binds to the a chain, calnexin is released and the class I molecule associates with the chaperone calreticulin and with tapasin
- ✓ Tapasin (TAP-associated protein) brings the TAP transporter into proximity with the class I molecule and allows it to acquire an antigenic peptide
- ✓ Now, the class I molecule displays increased stability and can dissociate from calreticulin and tapasin, exit from the RER


(b)
Cytosol
Protein
Amino acids
Peptides
ATP
ADP + Pi
TAP
Class
I MHC
Tapasin
Calreticulin
RER
lumen
Class I α chain
Calnexin


β2 microglobulin
+ Peptides
Exit RER
Class I MHC α chain
Calnexin
Calnexin-associated class I MHC α chain
Calreticulin-tapasin-associated class I MHC molecule
Class I MHC molecule
Tapasin
Calreticulin
Calnexin
Tapasin
Calreticulin

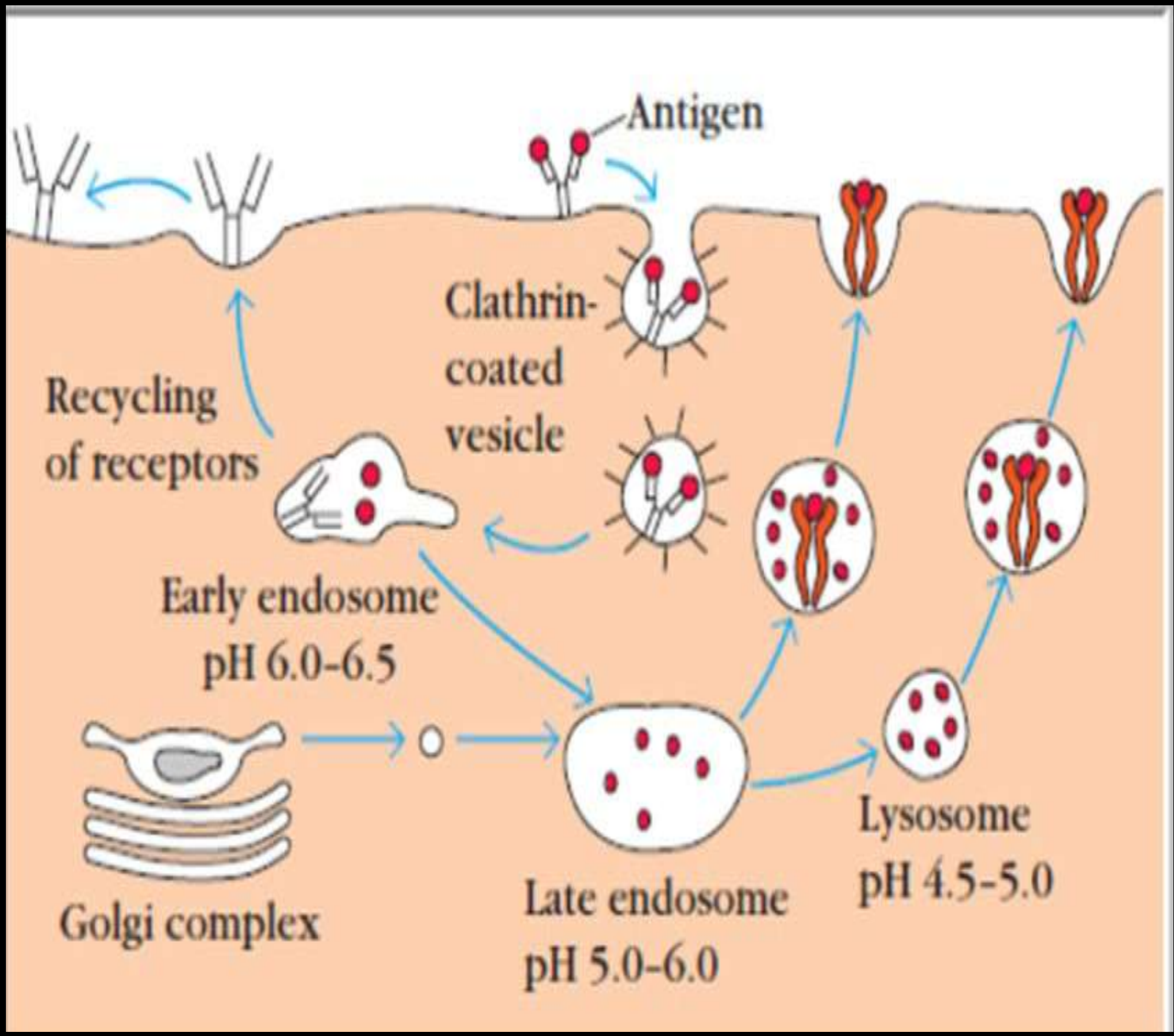
Endocytic Pathway

- Peptide Generation

  1) An antigen is internalized by antigen-presenting cells it is degraded into peptides within compartments of the endocytic processing pathway

  2) Internalized antigen moves from early to late endosomes and finally to lysosomes, encountering hydrolytic enzymes and a
  lower pH in each compartment

  3) Antigen is degraded into oligo peptides of about 13-18 residues, which bind to class II MHC molecules

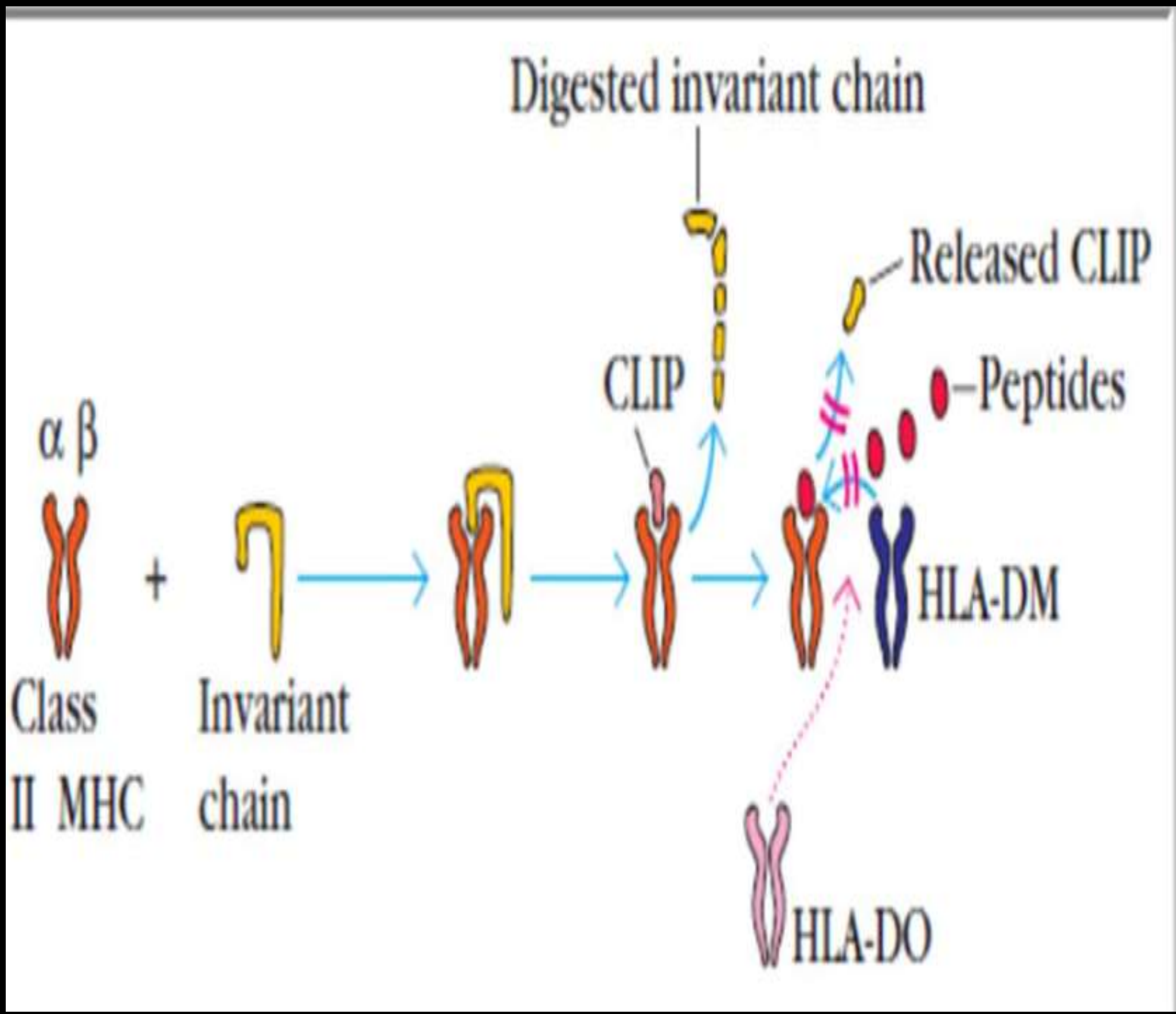

Antigen
Clathrin-
coated
vesicle
Recycling
of receptors
Early endosome
pH 6.0-6.5
Golgi complex
Late endosome
pH 5.0-6.0
Lysosome
pH 4.5-5.0

Transport of Class II MHC to Endocytic Compartment

- Class II MHC a and ß bind invariant chain (trimeric protein)
- Interacts with the peptide-binding cleft of the class II molecules, preventing any endogenously derived peptides from binding to the cleft while the class II molecule is within the RER
- Also appears to be involved in the folding of the class II MHC
- Invariant chain helps in exit of Class II MHC from the RER. and the subsequent routing of class II molecules to the endocytic processing pathway from the trans-Golgi network

Peptide assembly with Class II MHC

- In the endocytic pathway, the invariant chain is gradually degraded by increasing proteolytic activity
- A short fragment of the invariant chain termed CLIP (for class II-associated invariant chain peptide) remains bound to the class II molecule in the peptide-binding groove
- A non classical class II MHC molecule called HLA-DM is required to catalyze the exchange of CLIP with antigenic peptides
- The peptide-class II complex is transported to the plasma membrane. where the neutral pH appears to enable the complex to assume a compact. stable form


Digested invariant chain
Released CLIP
CLIP
Peptides
α β
+
HLA-DM
Class II MHC
Invariant chain
HLA-DO

THANKS!