ANTIGEN PROCESSING AND PRESENTATION

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DEFINITIONS

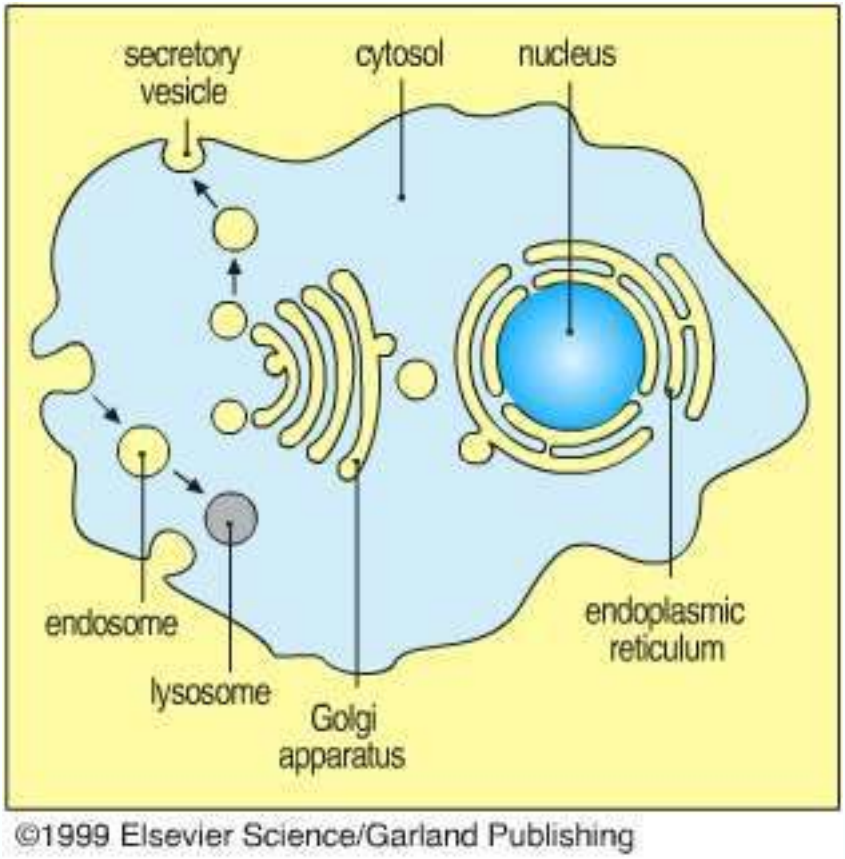
Antigen processing:

Proteolytic cleavage of proteins by enzymes (proteases) into small fragments (antigen peptides) and their association with MHC molecules by the antigen presenting cells. This is an active process requiring energy

Antigen presentation:

Presentation of processed peptides in association with MHC molecules (pMHC) on the surface of processing cells.

Antigen Processing Pathways
(Exogenous Antigens vs Endogenous Antigens)



- Endogenous proteins are processed in cytosol or in secretory vesicles and presented on class I MHC molecules to $CD8^+$ T cells.

- Exogenous proteins are processed in endosomes and presented on class II MHC molecules to $CD4^+$ T cells

Antigen Processing Pathways
(Exogenous Antigens vs Endogenous Antigens)

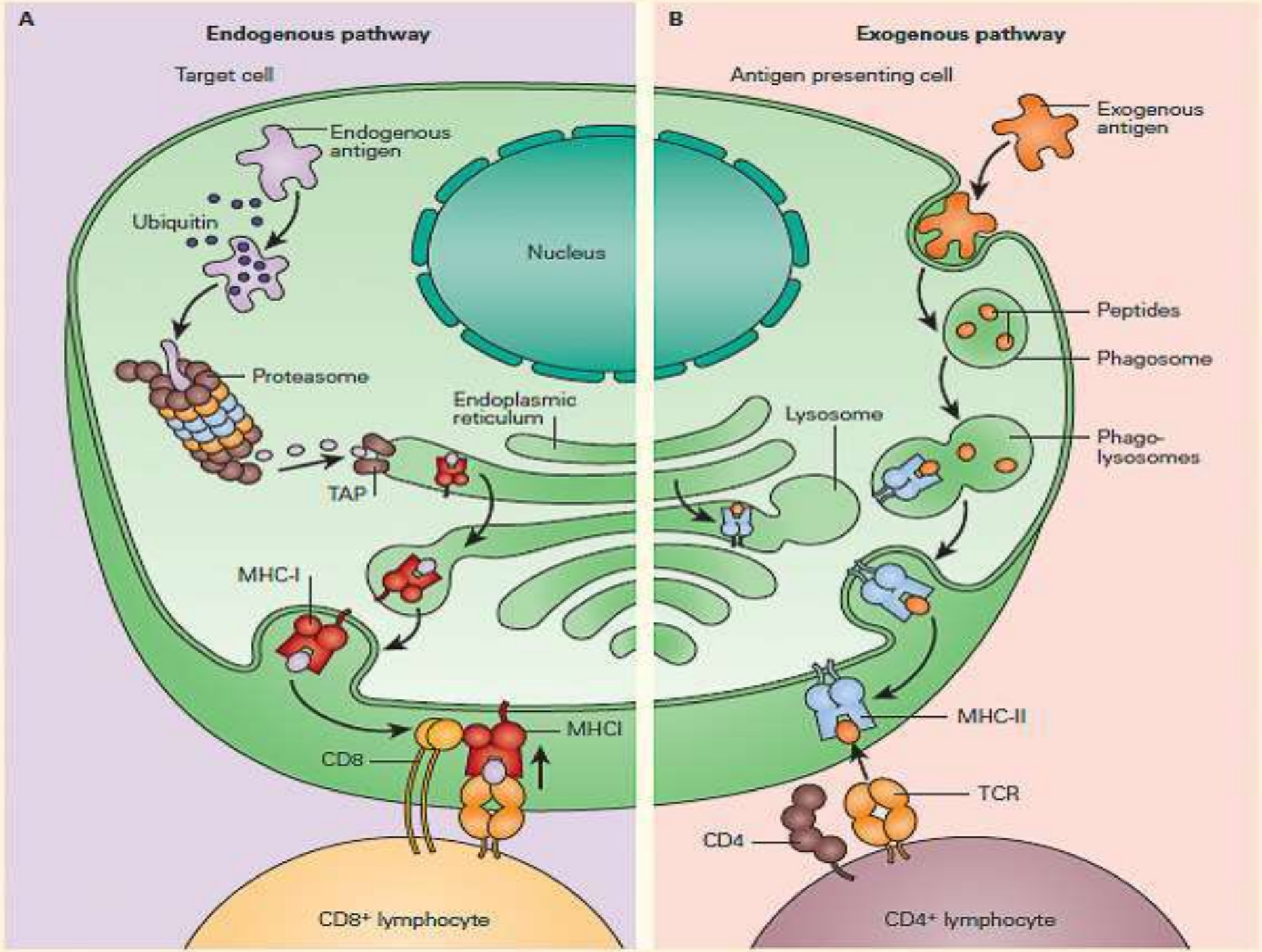
Endogenous Antigens

- Endogenous antigens are derived from proteins produced inside the cell.
- These includes altered self-protein antigens (e.g. tumor antigens) and non-self protein antigens (e.g. viral antigens).
- Endogenous antigens associate with Class I MHC molecules that activate cytotoxic $CD8^+$ T cells for killing infected cells and tumor cells (target or effector cells).
- Endogenous antigens can be processed and presented by any nucleated cell.

Antigen Processing Pathways
(Exogenous Antigens vs Endogenous Antigens)

Exogenous Antigens

- Exogenous antigens are derived from proteins produced outside the cell.
- These includes various bacterial, viral, protozoal, fungal and parasitic antigens which are derived from outside the body
- Exogenous antigens associate with Class II MHC molecules that activate helper $CD4^+$ T cells for providing help to B and Tc cells.
- Exogenous antigens are processed and presented by APCs


A
Endogenous pathway
Target cell
Endogenous antigen
Ubiquitin
Nucleus
Proteasome
Endoplasmic reticulum
TAP
MHC-I
MHCI
CD8
CD8+ lymphocyte
B
Exogenous pathway
Antigen presenting cell
Exogenous antigen
Peptides
Phagosome
Lysosome
Phago-lysosomes
MHC-II
TCR
CD4
CD4+ lymphocyte

Endogenous Antigens Processing Pathway
(Cytosolic Pathway)

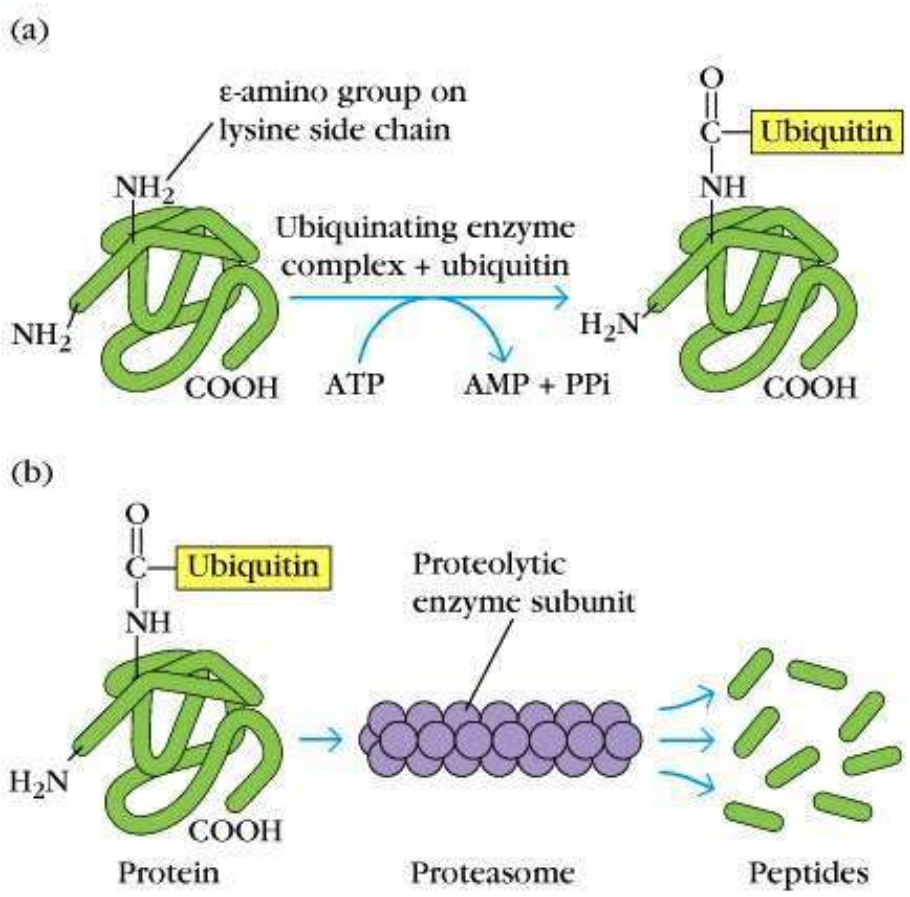
Endogenous (MHC class I) pathway

1. **Processing of antigens into peptides**
2. **Assembly of MHC and peptide loading complex**
3. **Peptide loading and MHC-peptide transport**

Endogenous Antigens Processing Pathway
(Step-Ia: Ubiquitination)

Covalent conjugation to Ubiquitin

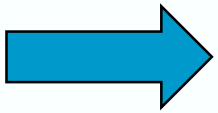
Ubiquitin targets proteins to Proteasome



Ubiquitin proteasome pathway for cytosolic protein degradation

Endogenous Antigens Processing Pathway
(Step-Ib: Proteasome-mediated processing)

The **proteasome** is a cylindrical shaped catalytic protease complex of 28 subunits for cytosolic protein degradation.

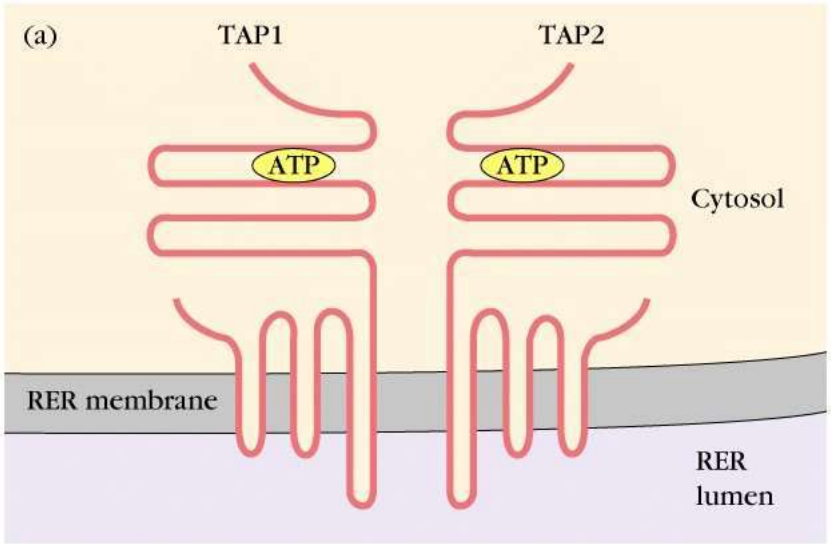


The proteasome unfolds proteins and then **cleaves proteins** into peptides and amino acids by proteases

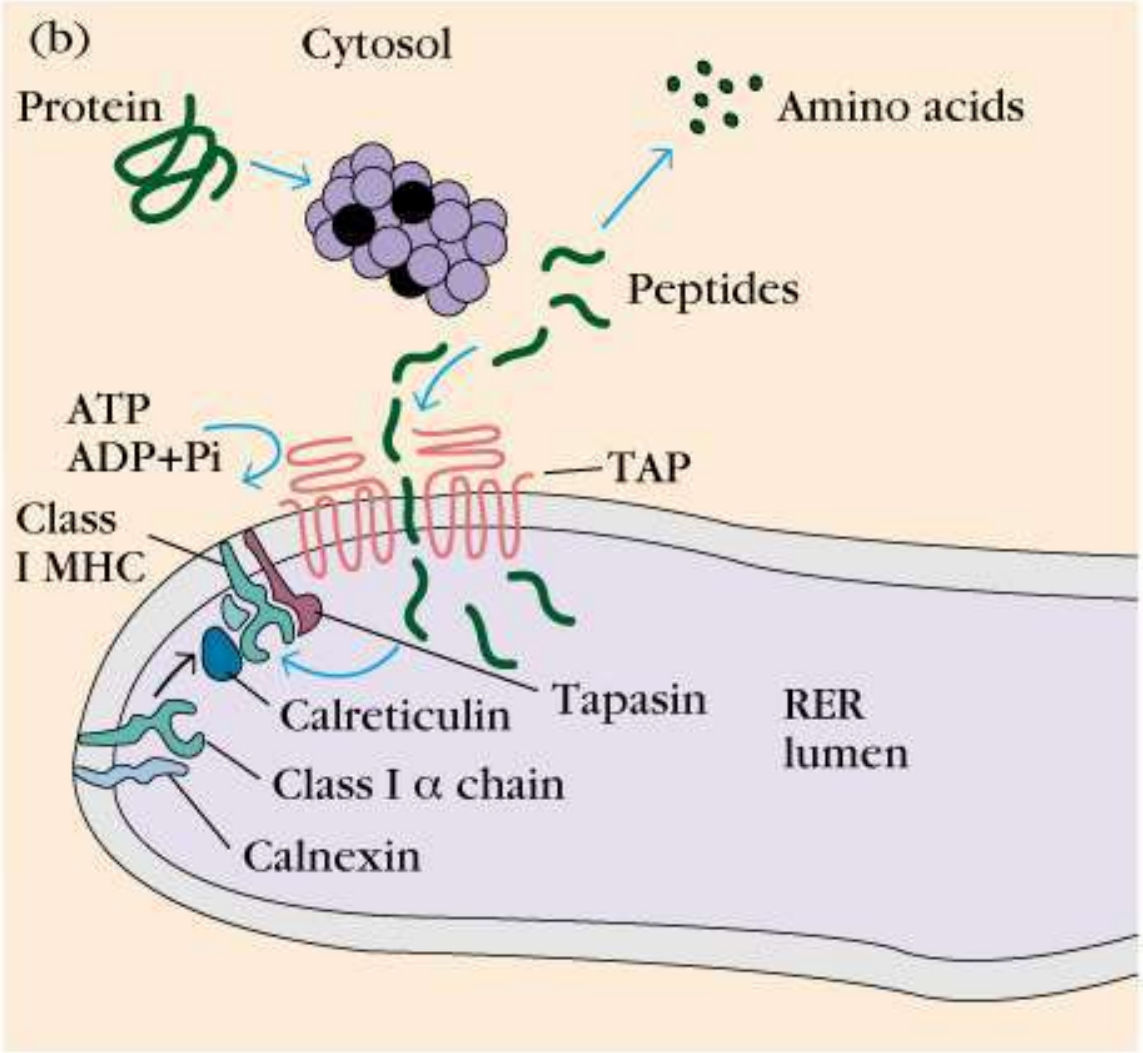
Conserved throughout the eukaryotes and the archaebacteria

Endogenous Antigens Processing Pathway (Step-II: Transfer of peptides by TAP proteins)

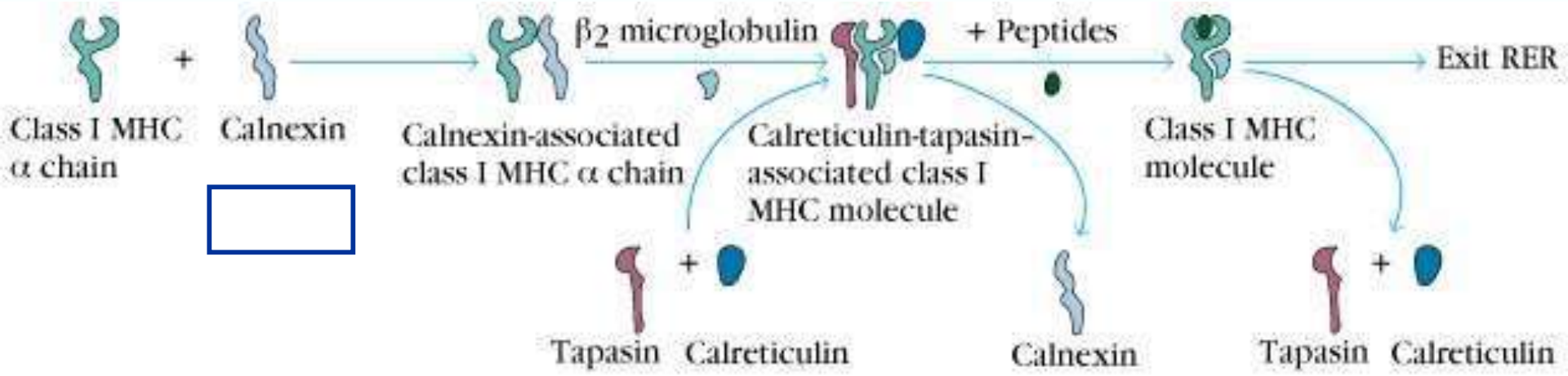
- **TAP proteins** (Transporters associated with Antigen Processing)
- TAP 1 and TAP 2 form heterodimer in membrane of ER to facilitate selective transport of peptides from cytoplasm into lumen of ER.
- TAP pump preferentially transport peptides with a length of 8–15 amino acids



Endogenous Antigens Processing Pathway
(Step-II: Peptides being transported by TAP proteins)



Endogenous Antigens Processing Pathway (Step-III: Generation of Class I MHC Peptides)



- Calnexin is a chaperone protein that binds to newly synthesized α-chain of Class I MHC and retains the Class I MHC from being degraded until β2-microglobulin binds.
- Tapasin and Calreticulin both bind to the newly formed Class I MHC complexes. Tapasin forms a bridge between the TAP proteins with the Class I MHC molecules, whereas calreticun prevents lodging of any other peptide in agerotope.

Endogenous Antigens Processing Pathway (Step-III: Association of Peptides with MHC)

- Peptides replaces tapasin and calreticulin and bind to the agerotope of Class I MHC molecules to form pMHC

- Peptide binding provides stability for Class I MHC to allow transfer to surface.

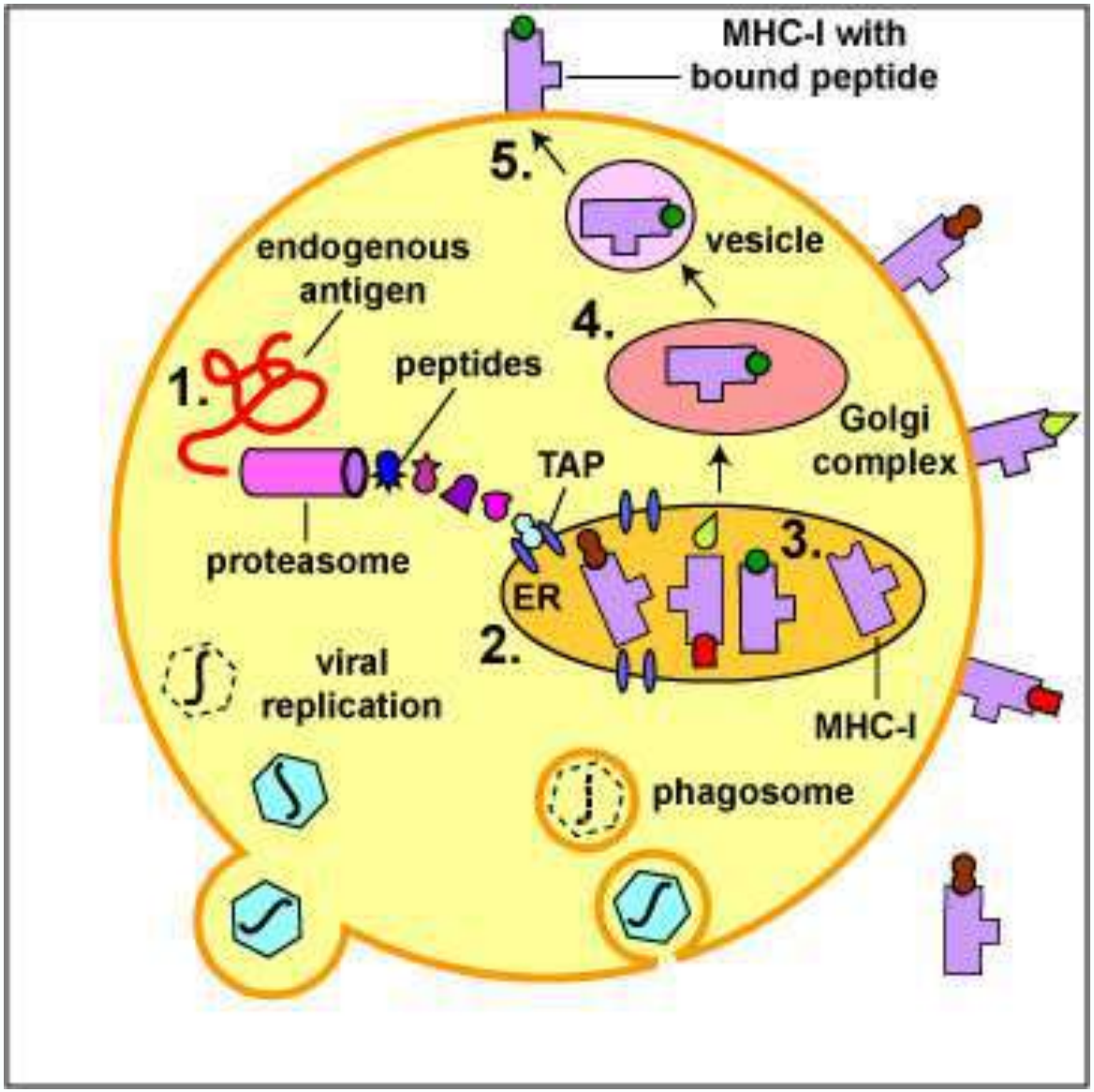
Endogenous Antigens Processing Pathway

(Step-IV and V:Transport of pMHC to cell surface and presentation)

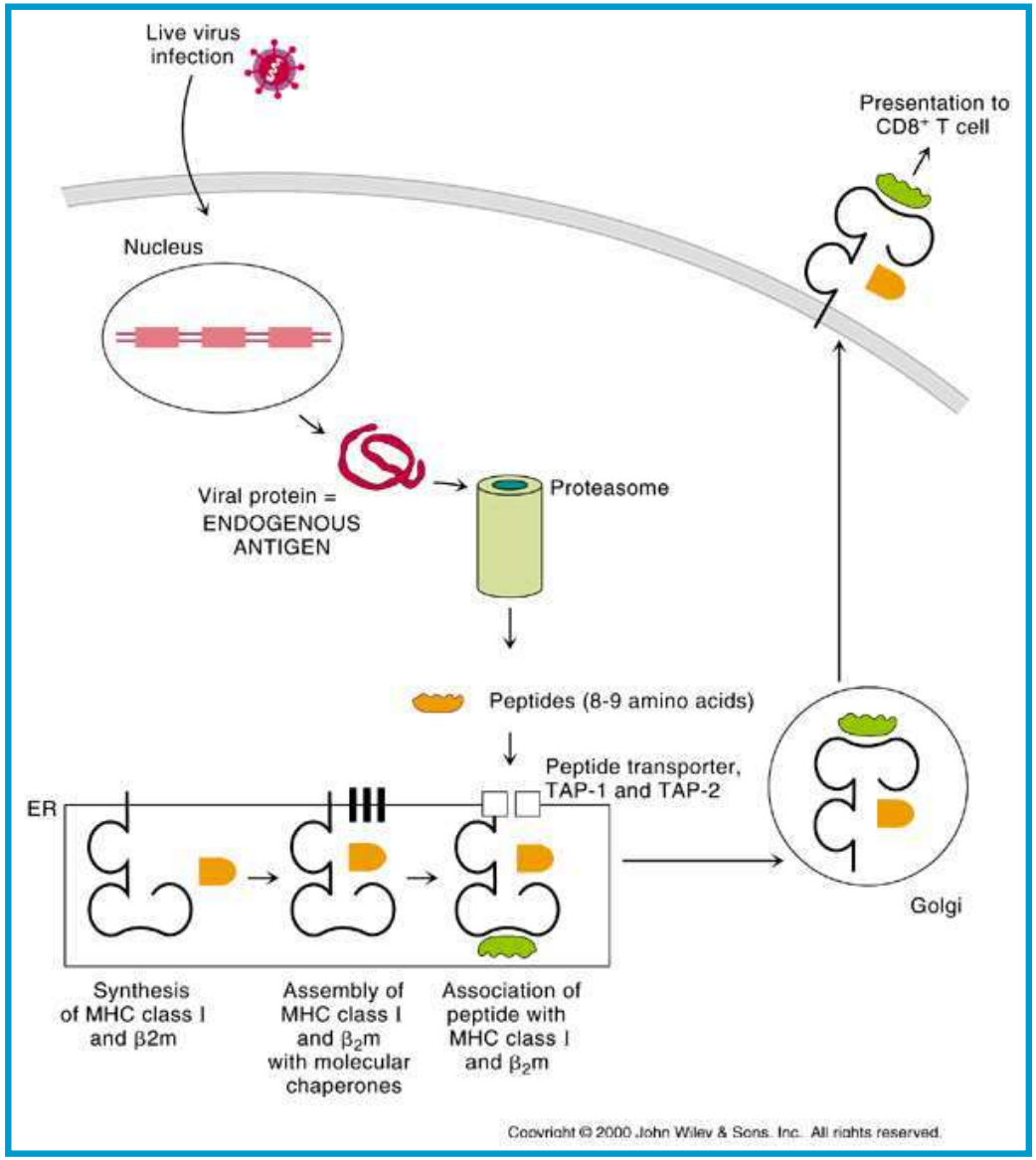
- The pMHC-I complex is transported from ER via Golgi bodies in a membrane bound vesicle to the cell surface.

- The membrane of transport vesicle fuse with the cell membrane and pMHC complex bind to membrane presenting peptide lodged in agerotope toward exterior to be recognised by Tc cell

Endogenous Antigens Processing Pathway



Endogenous Antigens Processing Pathway



Endogenous Antigens Processing Pathway
(Peptide Trimming after Proteasome cleavage)

- Though a majority of peptides are ready after leaving proteasome to be transported to ER, upto 15% still need trimming.

- Cytosolic proteases have been identified that can trim NH_2 terminal after proteasome cleavage .

- Recent data indicate that peptides can be also be trimmed in ER to fit in Class I MHC pocket.

Exogenous Antigens Processing Pathway
(Endocytic Pathway)

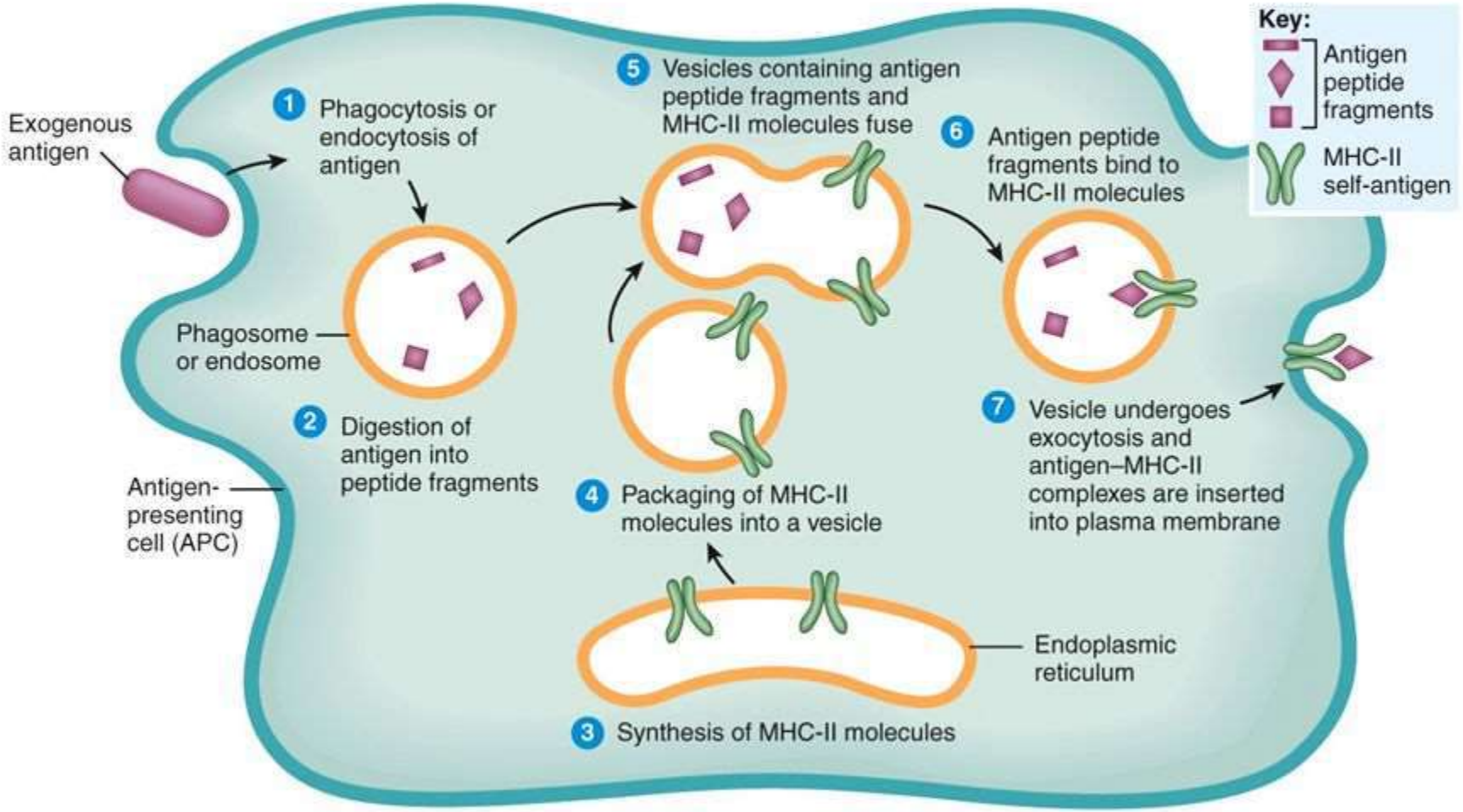
Exogenous (MHC class II) pathway

1. **Uptake and processing of exogenous antigen**

2. **MHC assembly and transport to peptide loading compartment**

3. **Peptide loading (CLIP exchange) and MHC-peptide transport**

Exogenous Antigen Processing



APCs present exogenous antigens in association with MHC-II molecules

Exogenous Antigens Processing Pathway (Step-I: How are peptides generated?)

- Peptides bound to MHC Class II molecules are derived from engulfed pathogens (also self proteins and internalized TM proteins)
- APCs internalize antigens by phagocytosis, by endocytosis, or both; macrophages internalize antigens by both mechanisms whereas dendritic cells and B cells internalize exogenous antigens by endocytosis into endosomes
- The exogenous antigen is degraded into peptides within these endocytic vesicles.
- Acidification of endocytic vesicles activates proteases that degrade proteins into fragments. The endocytic vesicles are highly acidic (low pH) and have more than 40 hydrolases that cut the antigen into peptides 13-18 amino acids long.
- These peptide fragments are to be loaded onto MHC class II molecules

Exogenous Antigens Processing Pathway
(Step-I: How are peptides generated?)

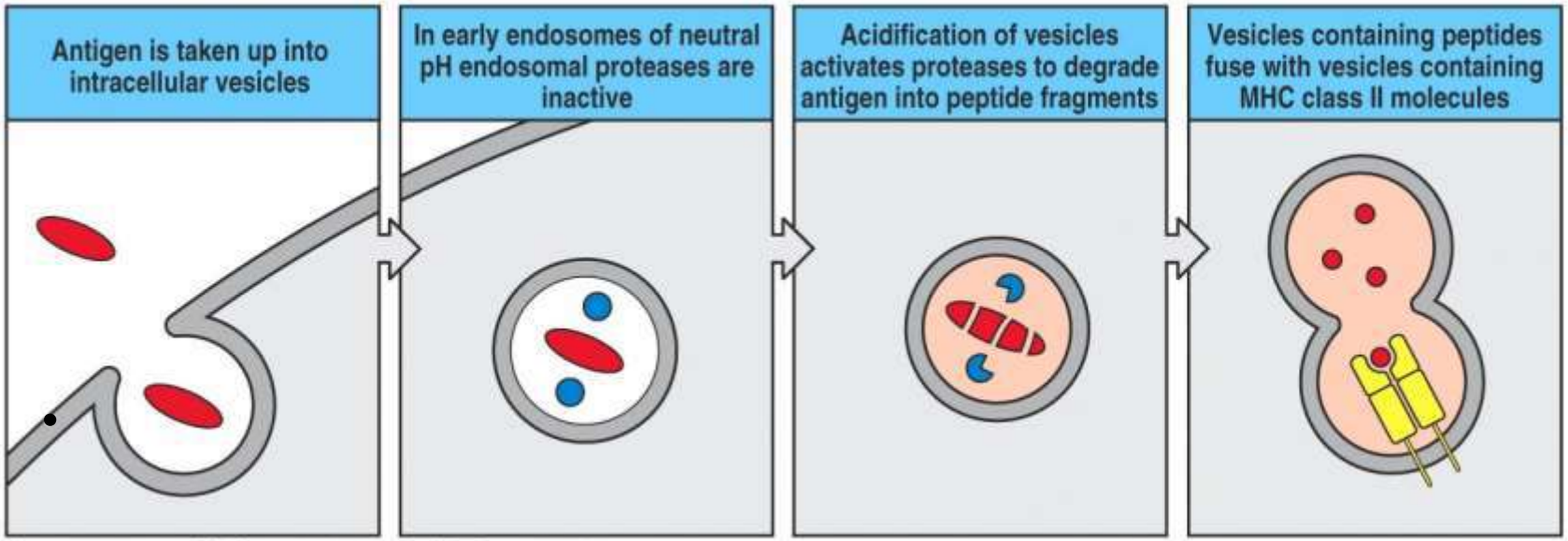


Figure 5-7 Immunobiology, 6/e. (© Garland Science 2005)

Exogenous Antigens Processing Pathway
(Step-II: Generation of MHC class II molecules)

- Class-II MHC molecules consist of two trans-membrane polypeptides (α and β) and a third molecule nestled in the groove they form.

- All three components of this complex must be present in the ER for proper assembly.

- A protein called the **invariant chain** ("**Ii**") temporarily occupies the groove till the antigenic peptides are not transported.

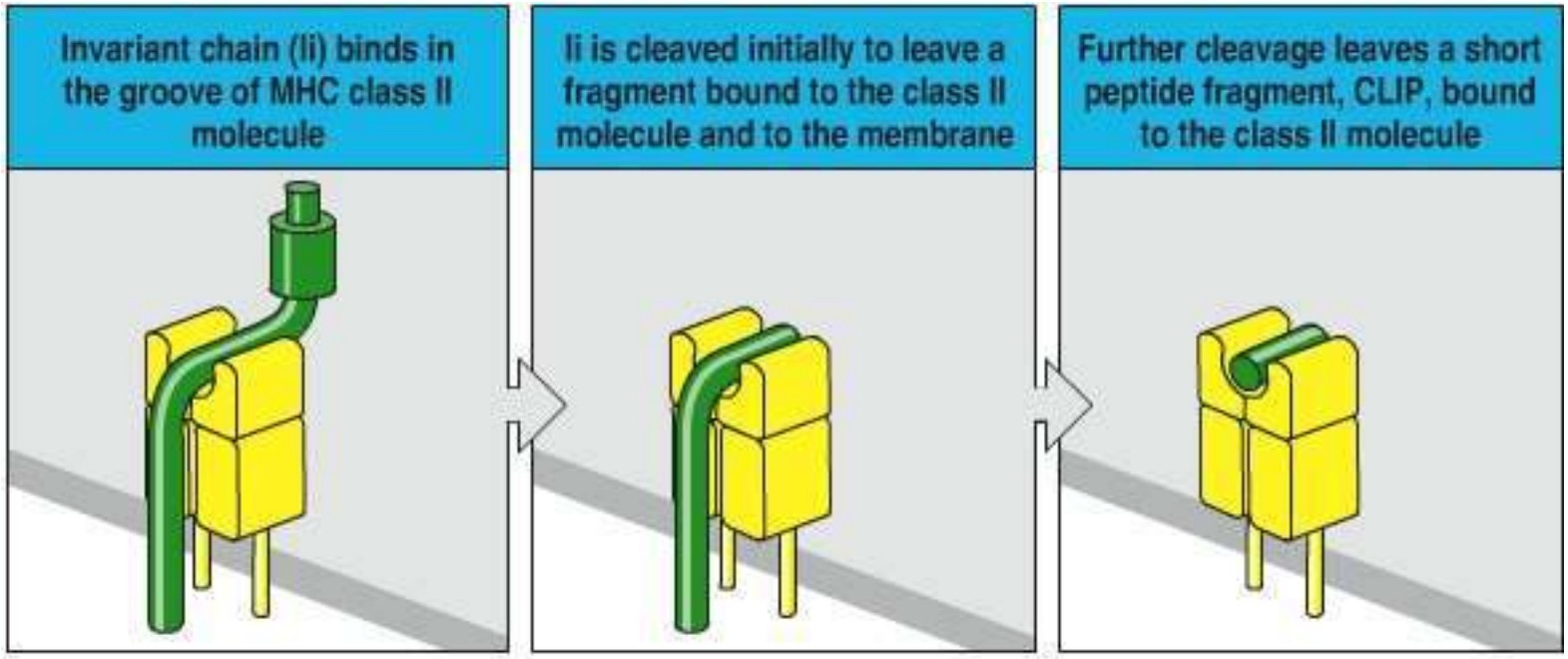
- **The steps:**
  - The two chains α and β of the class II molecule associate into the membrane of the ER.
  - They bind one molecule of **Ii** in groove.
  - This trimolecular complex is transported through the Golgi apparatus and the trans golgi network into specialised vesicles.
  - These specialised vesicles deliver MHC class II to specialized compartments where peptide loading occurs

The Invariant chain (Ii)

- **Invariant chain (Ii)** binds to Class II MHC molecules in ER to prevent endogenous peptide binding.
- Also, the invariant chain transports the MHC class II molecule from the Golgi apparatus to the endocytic compartments.
- Signals in the cytoplasmic tail of Ii lead to proper sorting of MHC class II.
- In the endocytic compartments Ii is cleaved to leave a peptide fragment (CLIP) in the binding groove.

CLIP (Class II associate Invariant chain Peptide).

Ii is cleaved to leave CLIP peptide in Class II MHC Groove



Invariant Chain Peptide (CLIP)

Exogenous Antigens Processing Pathway (Step-III: Class II MHC Peptide Loading)

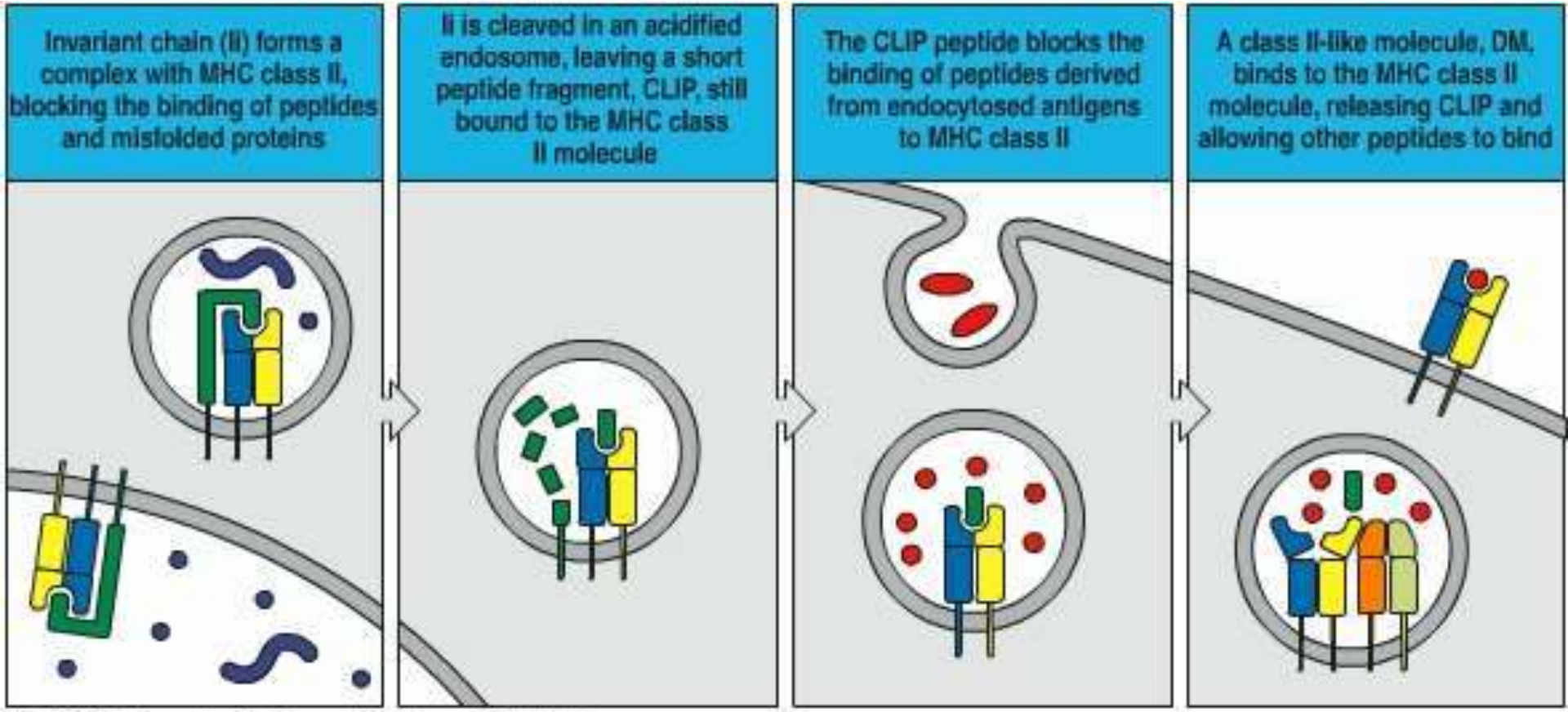
- Class II MHC molecule with Ii is transported to endosomes where processed peptides are present for loading into its groove .
- In the endocytic compartment Ii is cleaved by proteases into a small fragment called as CLIP.
- CLIP prevents premature binding of peptides to MHC class II molecules.
- A non-classical MHC class II molecule, called MHC-DM, removes CLIP from the peptide-binding cleft and helps to **load the antigenic peptide** into the groove (agerotope) of nascent MHC class II molecule to form pMHC
- Acidic pH is required for exchange of peptides.

(Chloroquine raise vesicular pH and block loading of Class II MHC)

HLA-DM

- HLA-DM (H-2M in mice) is a non-classical Class II like MHC molecule that binds to and stabilizes empty Class II molecules.
- HLA-DM helps in the release of CLIP fragment so that antigenic peptide can bind.
- MHC-DM is only expressed in the membranes of the endocytic vesicles.
- The peptide exchange is inhibited by another non-classical MHC class II molecule, called MHC-DO

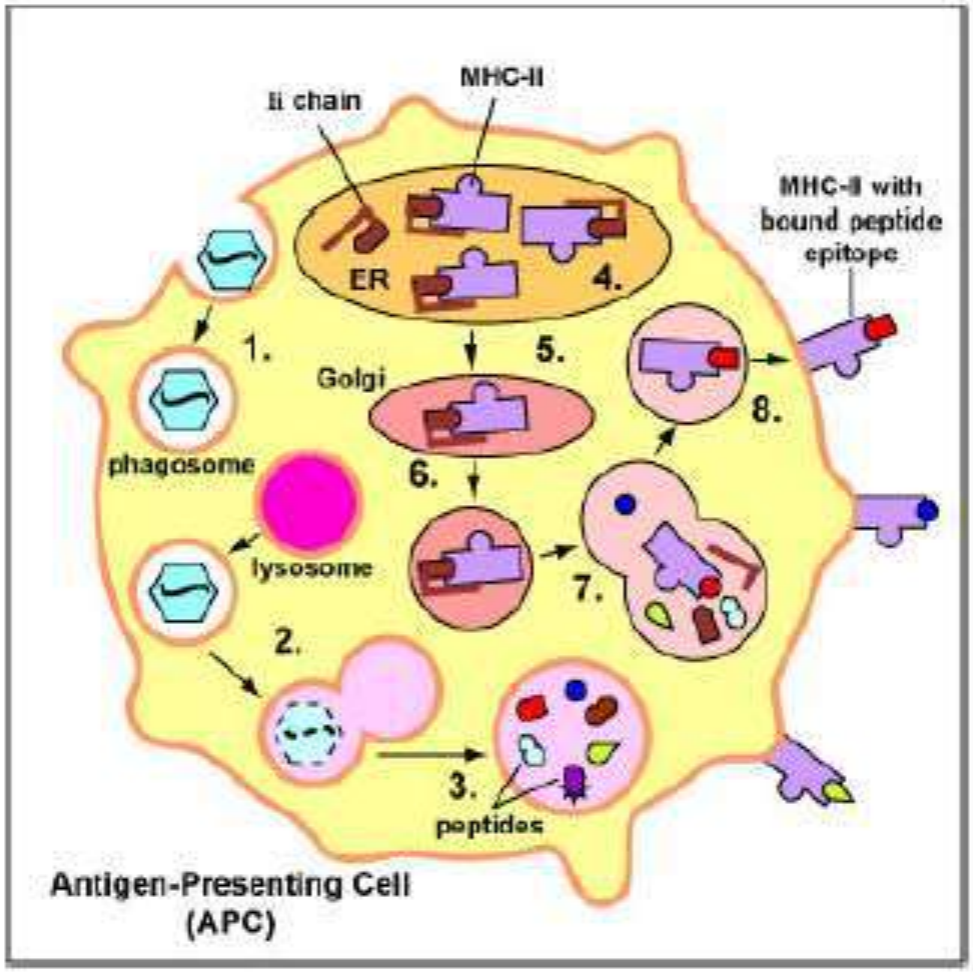
Ii Chain Prevents Newly synthesized self proteins from binding Class II MHC groove until Class II MHC is in endosomes.



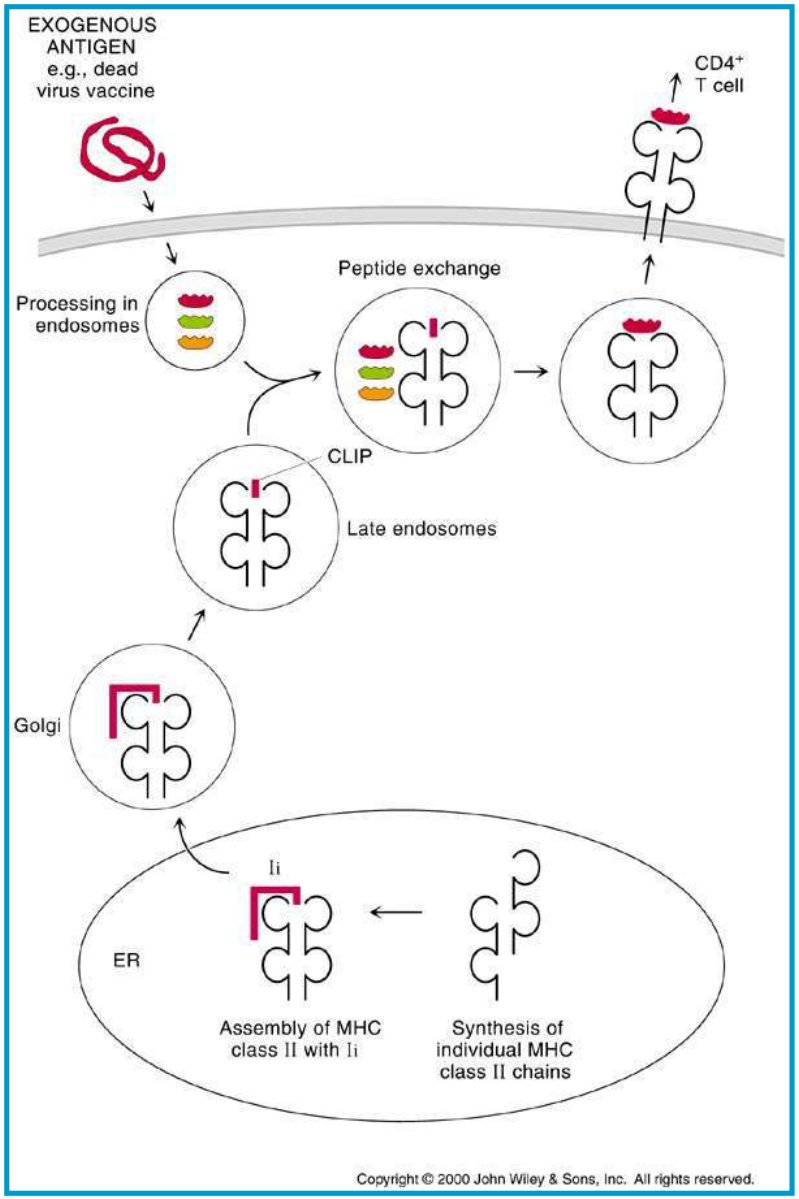
Exogenous Antigens Processing Pathway (Step-IV: MHC-peptide transport)

- The peptide loaded Class II MHC molecule – pMHC is transported into a membrane bound vesicle to the plasma membrane.
- The membrane of transport vesicle fuse with the cell membrane and pMHC complex bind to membrane and displayed at the cell surface
- It is presented to Th cells with appropriate TCR and CD4 molecules

Exogenous Antigens Processing Pathway



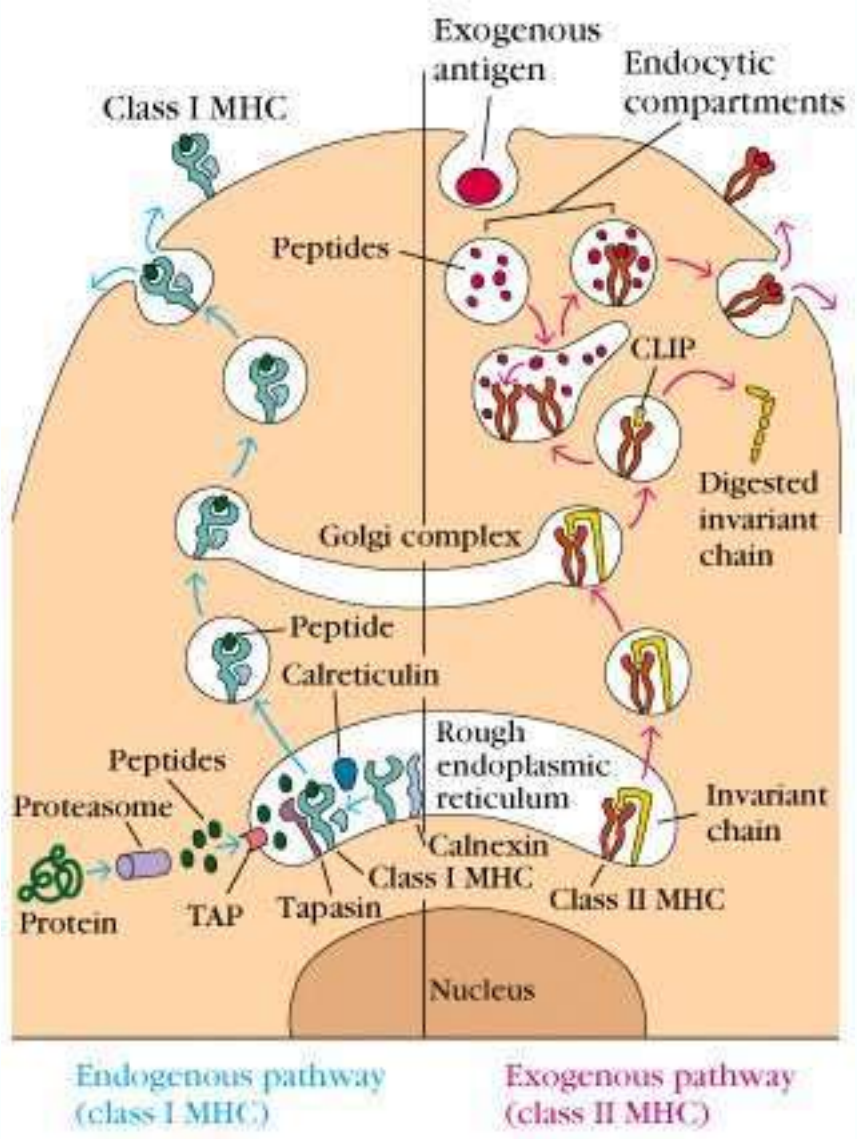
Exogenous Antigens Processing Pathway



Exogenous versus Endogenous pathways of Ag processing

Feature	Exogenous Pathway	Endogenous Pathway
Type of MHC	Class II	Class I
Source of Ag	Exogenous	Endogenous
Types of APC	DC, MO, B cells	All nucleated
Responsive T cell	CD4 T cells	CD8 T cells
Cellular compartment	Endosome	Cytosol
Enzymes responsible For peptide degradation	Endosomal and lysosomal proteases	Cytosolic proteasome
Molecules involved in Transport of peptides and Loading of MHC molecules	Invariant chain (Ii), HLA-DM	TAP

Comparison of Pathways



How are T cell antigens kept apart?

- Class I and Class II MHC molecules both traverse through ER to cell surface but load peptides in different cell compartments.

Control is through accessory proteins

- Class I requires TAP, Tapasin etc as control.
- Class II requires low pH for removal of Ii.

Interconnections Between the Class I and Class II Pathways

Cross presentation of Antigens

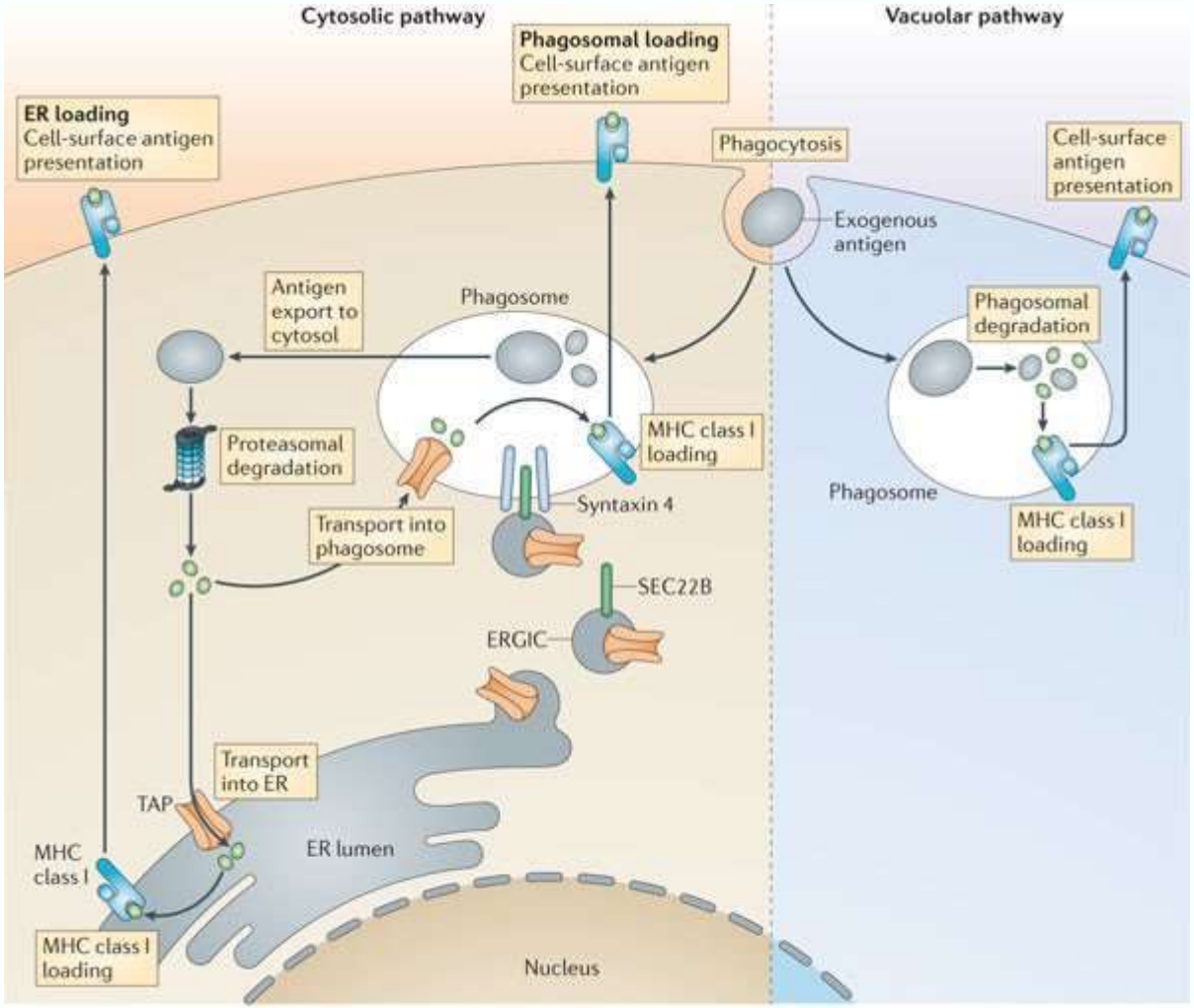
- The presentation of exogenous antigens on MHC class I molecules is known as **cross-presentation.**
- It is essential for the initiation of $CD8^+$ T cell responses.
- *In vivo*, cross-presentation is mainly carried out by specific dendritic cell (DC) subsets through an adaptation of their endocytic and phagocytic pathways ; the cDC1 are generally considered to be potent cross-presenting DCs *in vivo*.
- This process is important for the generation of an immune response against viruses and tumors, after vaccinations or in the induction of immune tolerance

Cross presentation of Antigens (contd.)

- Cross-presentation following infection by viruses is important because:
  - Most viruses infect cells other than APCs
  - While viral antigens displayed on the surface of any infected cell can serve as **targets** for cytotoxic T cells (CTLs), the lack of any costimulatory molecules on these cell surface makes them **poor stimulants** for the development of clones of CTLs, especially naïve cells, in the first place.
  - To become effector cytotoxic T lymphocytes (CTLs), naive CD8+ T cells need first to be activated by 'professional' antigen-presenting cells (APCs).
  - When the APCs are not directly infected, they need to acquire exogenous antigens from the infectious agent and present them on MHC class I molecules, which takes place by **cross-presentation.**

Major Pathways of Antigen Cross-Presentation

- In general, there are two main cross-presentation pathways: the vacuolar pathway and the endosome-to-cytosol pathway:
  - In the **vacuolar pathway**, antigen processing and loading onto MHC I molecules occurs within the endo/lysosomal compartment.
  - In the **endosome-to-cytosol pathway**, internalized antigens need to be transported from the endosomal compartment into the cytosol, where they are degraded by the proteasome and transported into the ER or back into the antigen-containing endosomes, where they can be loaded onto MHC class I



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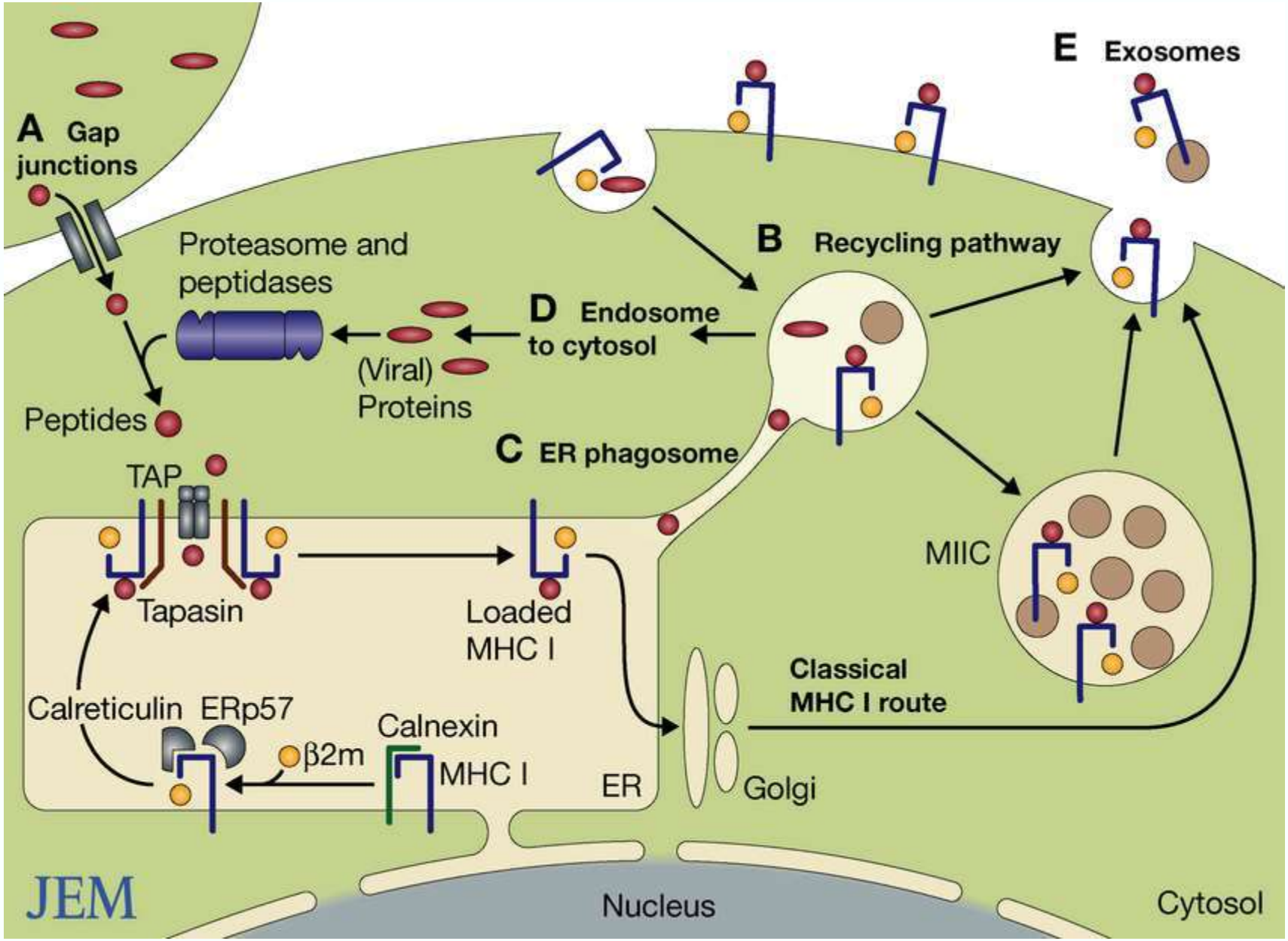
Alternate Pathways of Antigen Cross-Presentation

- In all cross-presentation pathways described above, cross-presented antigens entered the DC via endocytosis. However, there are some reports indicating that also distinct mechanisms can lead to cross presentation
- One of these mechanisms is the transport of pre-processed antigens (peptides) from a donor cell to a DC.
- Such transport can occur via direct cell–cell contact, mediated by gap junctions. After gap junction-mediated transport from one cell to another, antigen-derived peptides can enter the normal MHC I presentation pathway.
- Interestingly, the donor cell does not need to be an antigen-presenting cell, offering the possibility that DCs can obtain such peptides directly from infected cells. ss-presentation.


A Gap junctions
E Exosomes
B Recycling pathway
Proteasome and peptidases
D Endosome to cytosol
(Viral) Proteins
Peptides
C ER phagosome
TAP
Tapasin
Loaded MHC I
MIIC
Classical MHC I route
Calreticulin
ERp57
β2m
Calnexin
MHC I
ER
Golgi
JEM
Nucleus
Cytosol

Diverting Antigens from the Class I to the Class II Pathway

- **Autophagy** (cells begin to cannibalize some of their internal macromolecules, e.g., proteins and even organelles, e.g. mitochondria for re-use of their components) provides a mechanism by which cells can transfer **endogenous** (intracellular) antigens into the class II pathway, for example:
  - self-proteins so as to be able to delete $CD4^+$ T cells with receptors capable of attacking them and thus potentially capable of causing autoimmunity.
  - proteins synthesized by an infecting virus. In this way viral infection can generate **$CD4^+$ T cells** as well as cytotoxic T cells ($CD8^+$).