Structure & Function of MHC molecules

In contrast to antibodies or B-cell receptors, which can recognize an antigen alone, T-cell receptors only recognize pieces of antigen that are positioned on the surface of other cells. These antigen pieces are held within the binding groove of a cell surface protein called the **major histocompatibility complex (MHC) molecule**, encoded by a cluster of genes collectively called the MHC locus. These fragments are generated inside the cell following antigen digestion, and the complex of the antigenic peptide plus MHC molecule then appears on the cell surface. MHC molecules thus act as a cell surface vessel for holding and displaying fragments of antigen so that approaching T cells can engage with this molecular complex via their T-cell receptors.

The MHC got its name from the fact that the genes in this region encode proteins that determine whether a tissue transplanted between two individuals will be accepted or rejected. George Snell introduced the term ***histocompatibility*** (***H***) antigen to describe antigens provoking graft rejection and demonstrated that, of all the potential H antigens, differences at the H-2 (i.e., antigen II) locus provoked the strongest graft rejection seen between various mouse strains. Far from representing a single gene locus, H-2 proved to be a large complex of multiple genes, many of which were highly polymorphic, hence the term ***major histocompatibility complex*** (***MHC***).

MHC plays a central role in-

1. Determining whether the transplanted tissue will be accepted as self (histocompatible)
2. Development of both humoral and cell mediated immune response i.e., T cell recognize antigen when it is associated only with MHC.

The MHC Locus Encodes Three Major Classes of Molecules

The major histocompatibility complex is a collection of genes arrayed within a long continuous stretch of DNA on chromosome 6 in humans and on chromosome 17 in mice. The MHC is referred to as the **human leukocyte antigen (HLA) complex** in humans and as the **H-2 complex** in mice, the two species in which these regions have been most studied. Although the arrangement of genes is somewhat different in the two species, in both cases the MHC genes are organized into regions encoding three classes of molecules.

Mouse H-2 complex

Complex	H-2						
MHC class	I	II		III		I	
Region	K	IA	IE	S		D	
Gene products	H-2K	IA αβ	IE αβ	C′ proteins	TNF-α Lymphotoxin-α	H-2D	H-2L*

*Not present in all haplotypes

Human HLA complex

Complex	HLA							
MHC class	II			III		I		
Region	DP	DQ	DR	C4, C2, BF		B	C	A
Gene products	DP αβ	DQ αβ	DR αβ	C′ proteins	TNF-α Lymphotoxin-α	HLA-B	HLA-C	HLA-A

Comparison of the organization of the major histocompatibility complex (MHC) in mouse and human

- **Class I MHC genes** encode glycoproteins expressed on the surface of nearly all nucleated cells; the major function of the class I gene products is presentation of endogenous peptide antigens to $CD8^+$ T cells.
- **Class II MHC genes** encode glycoproteins expressed predominantly on APCs (macrophages, dendritic cells, and B cells), where they primarily present exogenous antigenic peptides to $CD4^+$ T cells.
- **Class III MHC genes** encode several different proteins, some with immune functions, including components of the complement system and molecules involved in inflammation.

There are two main classes of MHC molecules: class I and class II. Both of these two MHC molecules are membrane-bound glycoproteins that are closely related in both structure and function. Both classes of MHC molecule have been isolated and purified, and the three-dimensional structures of their extracellular domains have been resolved by x-ray crystallography. These membrane glycoproteins function as highly specialized antigen-presenting molecules with grooves that form unusually stable complexes with peptide ligands, displaying them on the cell surface for recognition by T cells via T-cell receptor (TCR) engagement. In contrast, class III MHC molecules are a group of unrelated proteins that do not share structural similarity or function with class I and II molecules, although many of them do participate in other aspects of the immune response.

Class I and class II molecules are very similar in their final quaternary structure, although they differ in how they create these shapes via primary through quaternary protein arrangements. MHC molecules assemble within the cell, where they associate with short peptide fragments derived either from proteins being made by the cell or proteins that have been internalized by the cell through phagocytosis or pinocytosis. So they also differ in terms of which cells express them and in the source of the antigens they present to T cells. Class I molecules are present on all nucleated cells in the body and specialize in presenting antigens that originate from the cytosol, such as viral proteins (MHC class I molecules bind to peptides derived from proteins being synthesized within the cell). These are presented to $CD8^+$ T cells, which recognize and kill cells expressing such intracellular antigens. In contrast, class II MHC molecules are expressed almost exclusively on a subset of leukocytes called **antigen-presenting cells (APCs)** and specialize in presenting antigens from extracellular spaces that have been engulfed by these cells, such as fungi and extracellular bacteria (MHC class II molecules bind to peptides derived from proteins made external to the cell). Once expressed on the cell surface, the MHC class II molecule presents the antigenic peptide to $CD4^+$T cells, which then become activated and go on to stimulate immunity directed primarily toward destroying extracellular invaders.

MHC class I molecules:

Structure:

- A single class I MHC molecule consists of a 45-kilodalton (kDa) **α chain** associated noncovalently with a 12-kDa **β_2-microglobulin** molecule.
- The α chain is organized into three external domains (α_1, α_2, and α_3), each approximately 90 amino acids long; a transmembrane domain of about 25 hydrophobic amino acids followed by a short stretch of charged (hydrophilic) amino acids; and a cytoplasmic anchor segment of 30 amino acids.
- The α chain is encoded by polymorphic genes, referred to as *HLA-A*, *HLA-B*, and *HLA-C* within the A, B & C regions of human HLA complex located on chromosome no 6 and *H-2 K*, *H-2D*, and *H-2 L* genes within K, D/L region of mouse H2 complex located on

  chromosome no 17, which can result in the expression of at least three different class I proteins in every cell.
- Its companion, β_2-microglobulin, is similar in size and organization to the α_3 domain. β_2-microglobulin does not contain a transmembrane region and is noncovalently bound to the MHC class I α chain.
- β_2-microglobulin is a protein encoded by a highly conserved gene located on a different chromosome.
- Sequence data reveal strong homology between the α_3 domain of MHC class I, β_2-microglobulin, and the constant-region domains found in immunoglobulin molecule, that's why MHC class I has been categorized within the immunoglobulin superfamily proteins.

Functions of different domains:

- The α_1 and α_2 domains interact to form a platform of eight antiparallel β strands spanned by two long α -helical regions to form a deep groove, or cleft, with the long α helices as sides and the β strands of the β sheet as the bottom. This *peptide-binding groove* (-25Å X 10Å X 11Å) is located on the top surface of the class I MHC molecule, and it is large enough to bind a peptide of 8 to 10 amino acids in a flexible, extended conformation.
- The α_3 domain and β_2-microglobulin are organized into two β pleated sheets each formed by antiparallel β strands of amino acids.
- The α3 domain appears to be highly conserved among class I MHC molecules and contains a sequence that interacts strongly with the CD8 cell surface molecule found on TC cells.
- All three molecules (class I α chain, β_2-microglobulin, and a peptide) are essential for the proper folding and expression of the MHC-peptide complex on the cell surface. In absence of the β_2-microglobulin, class I MHC α chain is not expressed on the membrane.

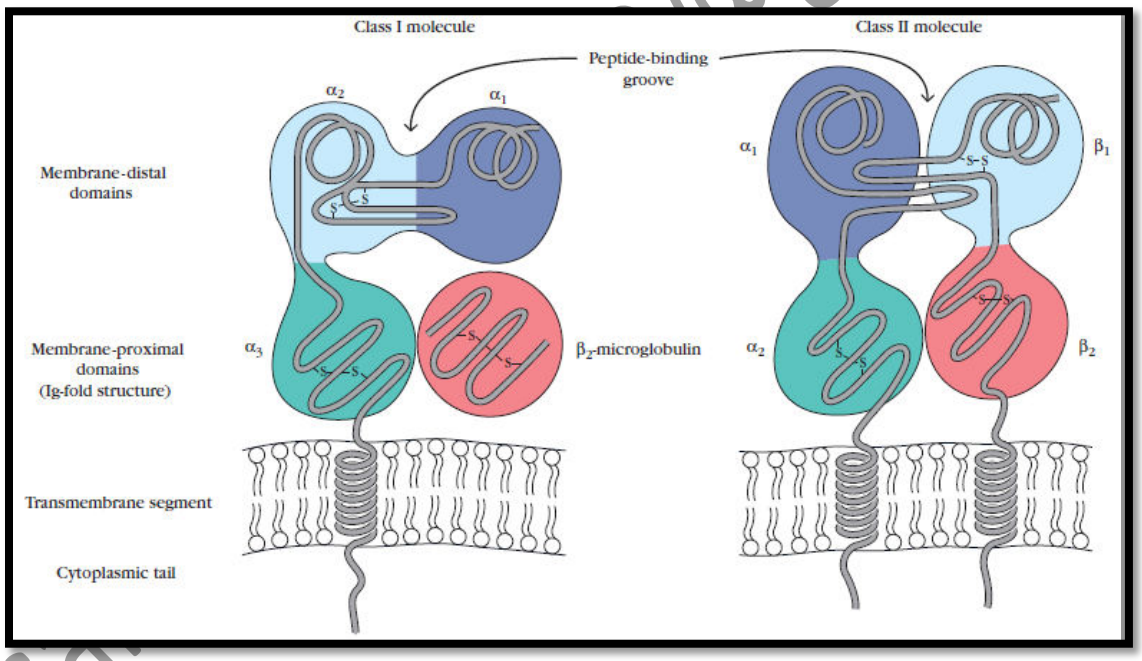

Fig: Schematic diagrams of class I and class II MHC molecules showing the external domains, transmembrane segments, and cytoplasmic tails

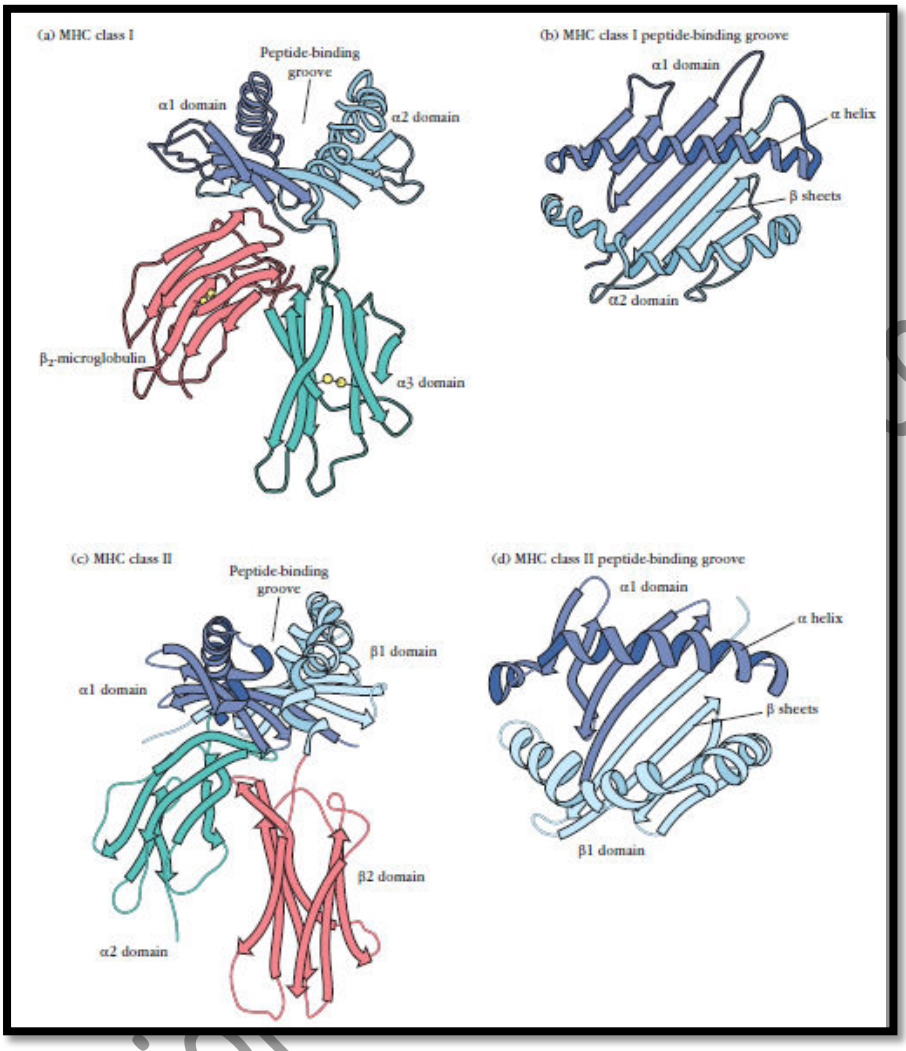

Fig: Representations of the three-dimensional structure of the external domains of human MHC class I and class II molecules based on x-ray crystallographic analysis

MHC class II molecules:

Structure:

- Class II MHC molecules contain two different polypeptide chains, a 33-kDa α chain and a 28-kDa β chain, which associate by noncovalent interactions.
- Like class I α chains, class II MHC molecules are membranebound glycoproteins that contain external domains, a transmembrane segment, and a cytoplasmic anchor segment.
- Each chain in a class II molecule contains two external domains: α_1 and α_2 domains in one chain and β_1 and β_2 domains in the other.
- The membrane-proximal α_2 and β_2 domains, like the membrane-proximal α_3/β_2-microglobulin domains of class I MHC molecules, bear sequence similarity to the

immunoglobulin-fold structure. For this reason, class II MHC molecules are also classified in the immunoglobulin superfamily.

- The membrane distal portions of class II molecule is composed of α_1 and β_1 domains and forms the peptide-binding groove for processed antigen.
- Three different types of MHC class II α-and β-chain genes expressed in humans, *HLA-DQ*, *HLA-DP*, and *HLA-DR*, and two pairs in mice, *H2-A* (I-A) and *H2-E* (I-E). Thus, humans can express a minimum of three different class II molecules.

Functions of different domains:

- The peptide-binding groove of class II molecules, like that found in class I molecules, is composed of a floor of eight antiparallel β strands and sides of antiparallel α helices, (Four strands of the floor of the cleft and one of the helices are formed by α_1 and the other four strands of the floor and the second helix is formed by β_1) where peptides typically ranging from 13 to 18 amino acids can bind.
- β_2 domain of class II MHC is the binding site for CD4.
- The polymorphic residues are located in α_1 and β_1, in and around the peptide binding cleft.
- A nonpolymorphic polypeptide called the invariant chain (I_i) is associated with newly synthesized class II molecule.

TABLE 8-1 Peptide binding by class I and class II MHC molecules

	Class I molecules	Class II molecules
Peptide-binding domain	α1/α2	α1/β1
Nature of peptide-binding groove	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	Conserved residues distributed along the length of the peptide
Nature of bound peptide	Extended structure in which both ends interact with MHC groove but middle arches up away from MHC molecule	Extended structure that is held at a constant elevation above the floor of the MHC groove

Different Antigen Processing and Presentation Pathways:

The immune system typically uses different pathways to eliminate intracellular and extracellular antigens. As a general rule, **endogenous antigens** (those generated within the cell) are processed in the **cytosolic** or **endogenous pathway** and presented on the membrane with class I MHC molecules. **Exogenous antigens** (those taken up from the extracellular environment by endocytosis) are typically processed in the **exogenous pathway** and presented on the membrane with class II MHC molecules.

Mechanism of Antigen processing & presentation by MHC class I molecule:

Endogenous antigen & cytosolic pathway:

1. Intracellular proteins are degraded into short peptides by a cytosolic proteolytic system present in all cells, called the proteasome. Many proteins are targeted for proteolysis when a small protein called **ubiquitin** is attached to them. These ubiquitin-protein conjugates enter the proteasome complex for degradation.
2. Each proteasome is composed of 14 subunits arrayed in a barrel-like structure of symmetrical rings. ubiquitin-protein conjugates enter the proteasome complex, consisting of the 20S base and an attached 19S regulatory component, through a narrow channel at the 19S end. The proteasome complex cleaves peptide bonds in an ATP-dependent process. Degradation of ubiquitin-protein complexes is thought to occur within the central hollow of the proteasome.
3. In addition to the standard 20S proteasomes resident in all cells, a distinct proteasome of the same size can be found in pAPCs and the cells of infected tissues. This distinct proteasome, called the **immunoproteasome**, has some unique components that can be induced by exposure to interferon-γ or TNF- α. *LMP2* and *LMP7*, genes that are located within the class I region and are responsive to these cytokines, encode replacement catalytic protein subunits that convert standard proteasomes into immunoproteasomes, increasing the production of peptides that bind effi ciently to MHC class I proteins.
4. Peptides generated in the cytosol by the proteasome are translocated by **TAP** (*t*ransporter associated with *a*ntigen *p*rocessing) which is a transporter protein, into the RER by a process that requires the ATP hydrolysis. **TAP** is a membrane-spanning heterodimer consisting of two proteins: TAP1 and TAP2. TAP has affinity for peptides containing 8 to 16 amino acids. The optimal peptide length for class I MHC binding is around 9 amino acids, and longer peptides are trimmed by enzymes present in the ER, such as **ERAP** (*e*ndoplasmic *r*eticulum *a*mino*p*eptidase).
5. The α chain and β2-microglobulin components of the class I MHC molecule are synthesized on ribosomes on the RER.
6. The assembly of these components into a stable class I MHC molecular complex involves several steps and includes the participation of *molecular* ***chaperones*** that facilitate the folding of polypeptides that can exit the RER. I also requires the presence of a peptide in the binding groove of the class I molecule.
7. The first molecular chaperone involved in class I MHC assembly is **calnexin**, a resident membrane protein of the ER. ERp57, a protein with enzymatic activity, and calnexin associate with the free class I α chain and promote its folding. When β_2-microglobulin binds to the α chain, calnexin is released and the class I molecule associates with the chaperone **calreticulin** and with **tapasin**.
8. Tapasin (*TAPas*sociated prote*in*) brings the TAP transporter into proximity with the class I molecule and allows it to acquire an antigenic peptide. The TAP protein promotes peptide capture by the class I molecule before the peptides are exposed to the luminal environment of the RER.
9. As a consequence of productive peptide binding, the class I molecule displays increased stability and can dissociate from the complex with calreticulin, tapasin, and ERp57. The class I molecule can then exit from the RER and proceed to the cell surface via the Golgi complex.

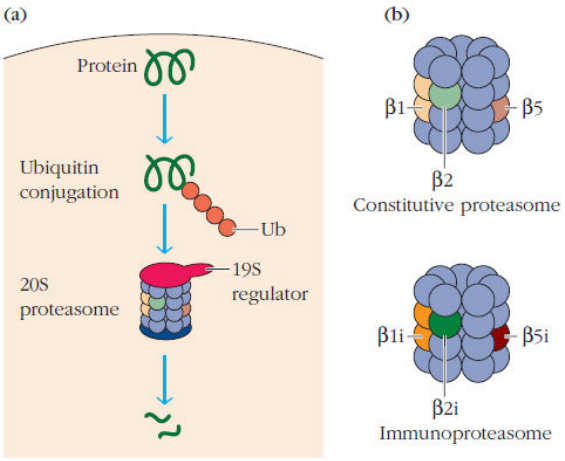

Fig: Cytosolic proteolytic system for degradation of intracellular proteins

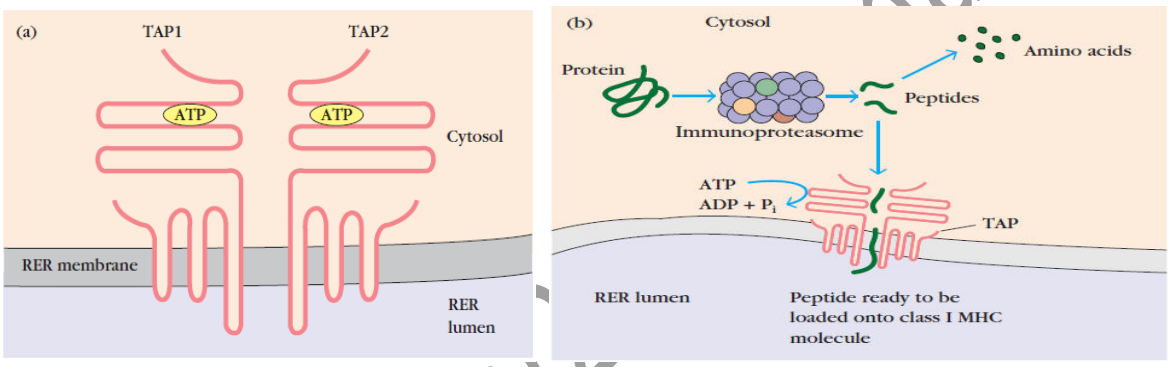

Fig: TAP (*t*ransporter associated with *a*ntigen *p*rocessing)

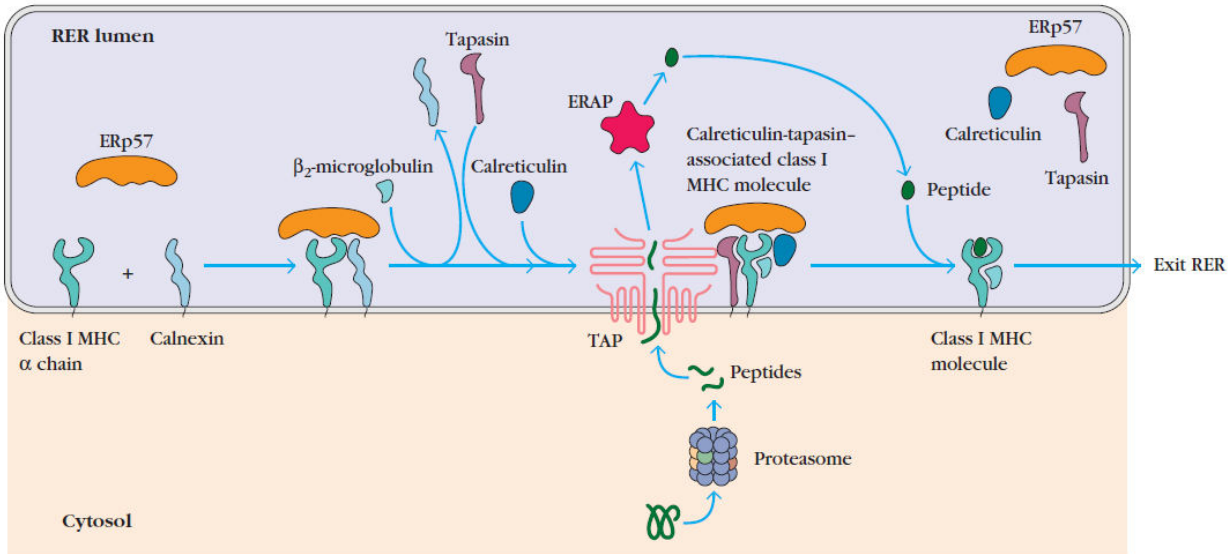

Fig: Assembly and stabilization of class I MHC molecules.

Mechanism of Antigen processing & presentation by MHC class II molecule:

EXogenous antigen & endocytic pathway:

1. Antigen presenting cells (APCs) can internalize particulate material by simple phagocytosis (also called "cell eating"), where material is engulfed by pseudopods of the cell membrane, or by receptor-mediated
2. endocytosis, where the material first binds to specific surface receptors. Macrophages and dendritic cells internalize antigen by both processes.
3. Once an antigen is internalized, it is degraded into peptides within compartments of the endocytic processing pathway.
4. The endocytic antigen processing pathway appears to involve several increasingly acidic compartments, including early endosomes (pH 6.0–6.5); late endosomes, or endolysosomes (pH 4.5–5.0); and lysosomes (pH 4.5). Internalized antigen progresses through these compartments, encountering hydrolytic enzymes and a lower pH in each compartment.
5. Antigen-presenting cells have a unique form of late endosome, the MHC class II-containing compartment (MIIC), in which final protein degradation and peptide loading into MHC class II proteins occurs. Within the compartments of the endocytic pathway, antigen is degraded into oligopeptides of about 13 to 18 residues that meet up with and bind to class II MHC molecules in late endosomes.
6. When class II MHC molecules are synthesized within the RER, these class II αβ chains associate with a protein called the **invariant chain** (**Ii**, **CD74**). This conserved, non-MHC encoded protein interacts with the class II peptide-binding groove preventing any endogenously derived peptides from binding while the class II molecule is within the RER.
7. The invariant chain also appears to be involved in the folding of the class II α and β chains, their exit from the RER, and the subsequent routing of class II molecules to the endocytic processing pathway from the trans-Golgi network. The invariant chain contains sorting signals in its cytoplasmic tail that direct the transport of the class II MHC complex from the trans-Golgi network to the endocytic compartments.
8. Recent experiments indicate that most class II MHC invariant chain complexes are transported from the RER, where they are formed, through the Golgi complex and trans-Golgi network, and then through the endocytic pathway, moving from early endosomes to the MIIC late endosomal compartments, and finally to lysosome.
9. As the proteolytic activity increases in each successive compartment, the invariant chain is gradually degraded. However, a short fragment of the invariant chain termed **CLIP** (for *cl*ass II–associated *i*nvariant chain *p*eptide) remains bound to the class II molecule after the majority of the invariant chain has been cleaved within the endosomal compartments
10. CLIP physically occupies the peptide-binding groove of the class II MHC molecule, preventing any premature binding of antigen-derived peptide.
11. A nonclassical class II MHC molecule called HLA-DM is required to catalyze the exchange of CLIP with antigenic peptides.The reaction between HLA-DM and the class II CLIP complex facilitating exchange of CLIP with another peptide is impaired in the presence of HLA-DO, which binds to HLA-DM and lessens the efficiency of the exchange reaction.

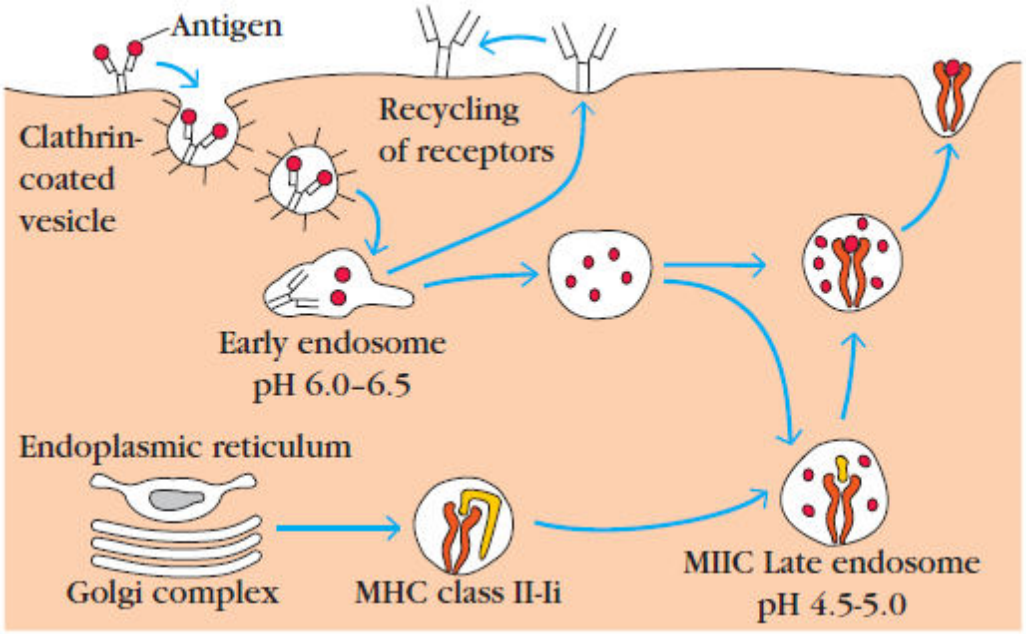

Fig: Generation of antigenic peptides in the exogenous processing pathway.

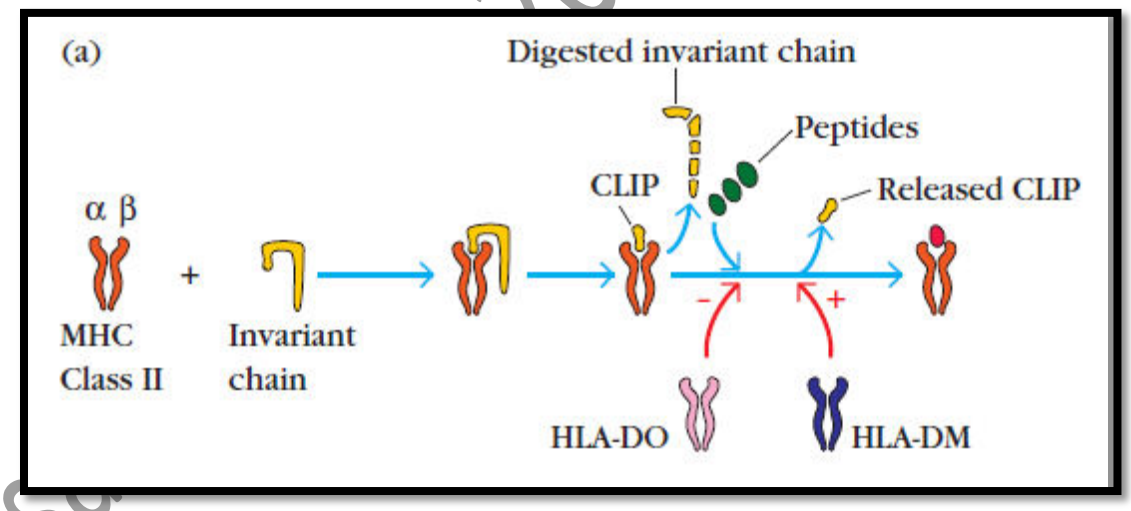

Fig: Assembly of class II MHC molecules.

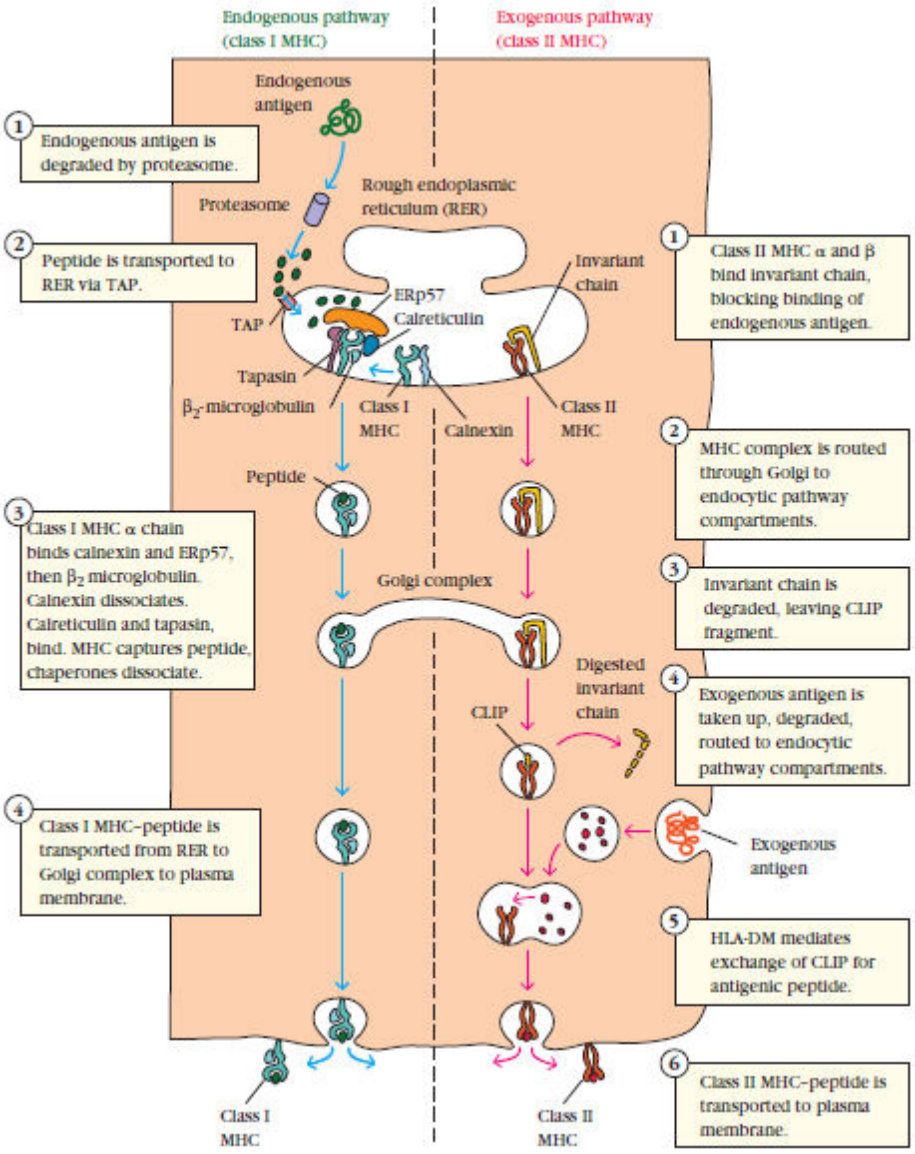

Fig: Separate antigen-presenting pathways are utilized for endogenous (green) and exogenous (red) antigens

References:

- **Kuby Immunology Seventh edition.**
- **Roitt's Essential Immunology Thirteenth edition**