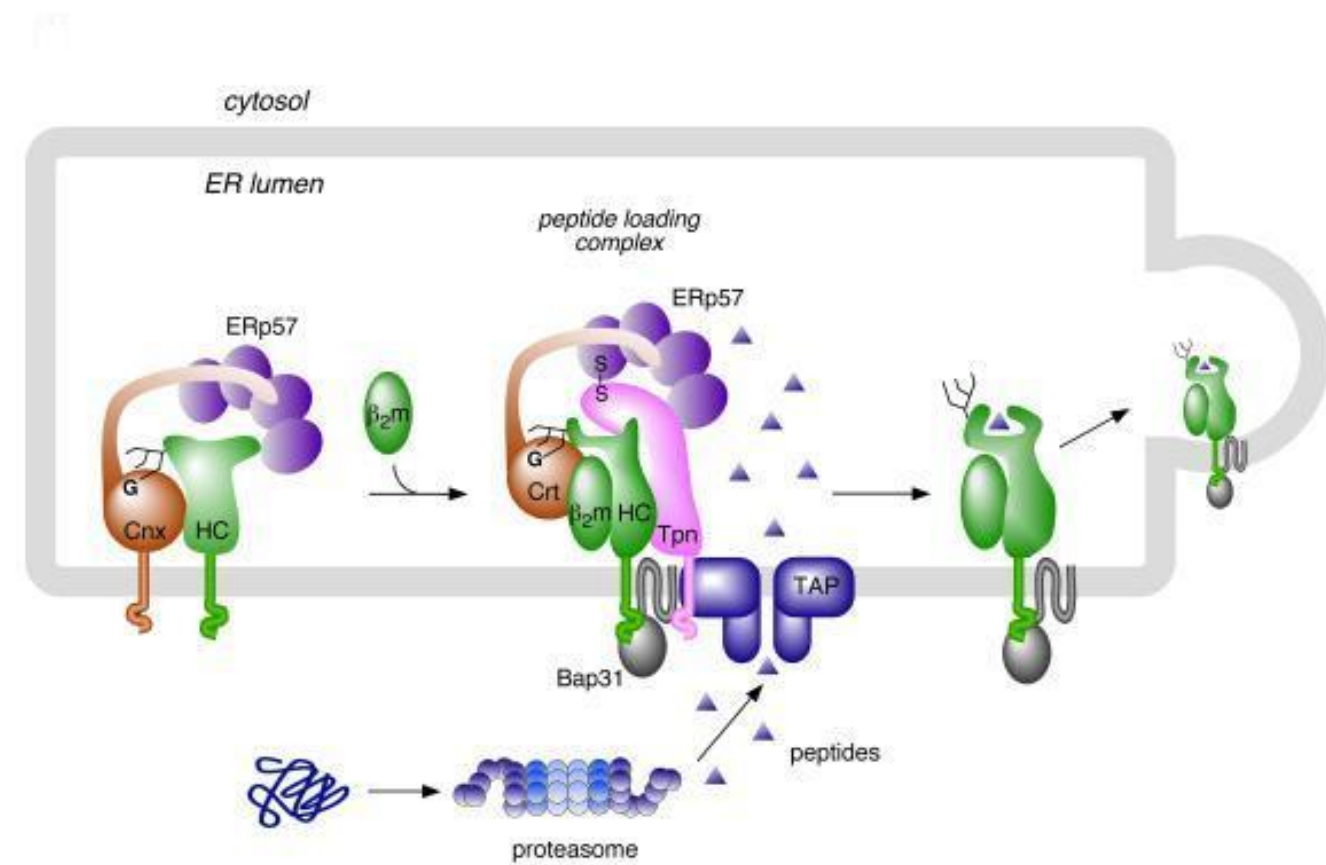


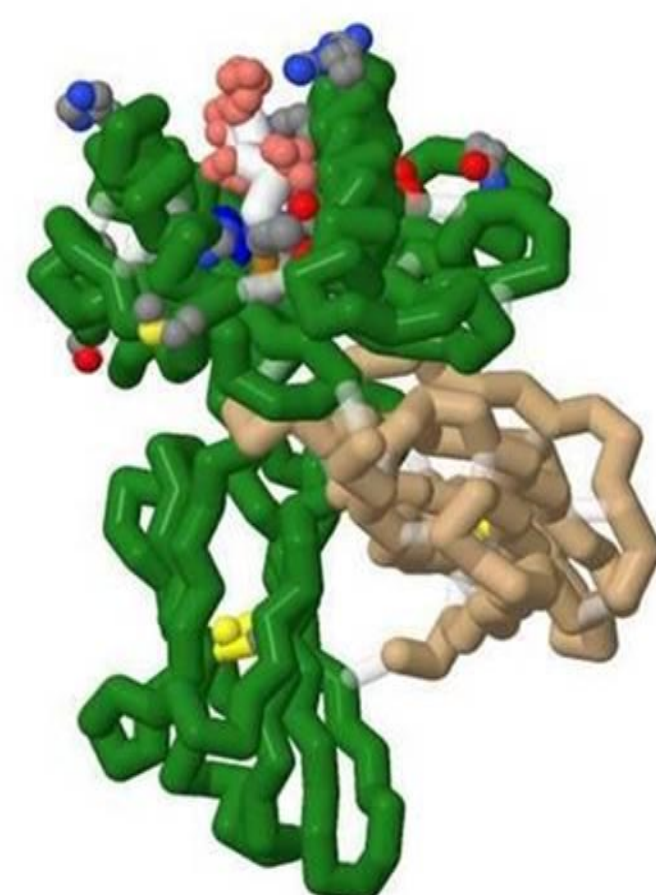
**Abstract:** The human immune system has developed the ability to fight many different infectious agents. Cytotoxic T lymphocytes (CTL) are cells of the immune system that recognize viral-infected cells by binding to portions of viral proteins (peptides) presented on class I major histocompatibility complex (MHC) proteins. MHC proteins are located in the cytoplasmic membrane of all nucleated cells and are composed of two protein chains, an alpha ( $\alpha$ ) chain and beta-2 microglobulin. The alpha chain is an integral membrane protein consisting of a transmembrane region, as well as three external immunoglobulin domains called  $\alpha$  1, 2, and 3. The base of the peptide-binding cleft is composed of an antiparallel beta-sheet formed between two alpha helices from the alpha-1 and 2 domains. Beta-microglobulin is loosely associated to the  $\alpha_3$  domain and is important for the stabilization of the class I MHC. Humans without  $\beta_2$ -microglobulin will not express class I MHC on the surface of their cells and are prone to recurring viral infections. Herpes viruses establish long-term latent infections achieved by evading class I MHC antigen presentation. Human herpesvirus-7 (HHV-7) produces a unique protein, U21, that blocks expression of human class I MHC, HLA-A, which prevents CTL recognition.

**Introduction:** By the age of 3, nearly 90% of the world's population is infected with Human herpesvirus-7 (HHV-7) which remains largely quiescent for the rest of the individual's life. HHV-7 is able to avoid detection by T-lymphocytes and Natural Killer (NK) cells in the human immune system by producing a protein, U21, that binds to class I MHC molecules and prevents their transport to the cytoplasmic membrane. Since the function of class I MHC is to present peptides from intracellular pathogens to CTLs, loss of MHC expression on the cell surface prevents immune recognition of viral-infected cells. Thus, many viruses have developed ways to prevent class I presentation. However, if MHC is completely missing from a cell surface, other immune cells, called Natural killer (NK) cells are activated to destroy the infected cell. The studies described here demonstrate the ability of U21 to attach to class I MHC in the RER and divert the protein to the lysosomal compartment of the cell.



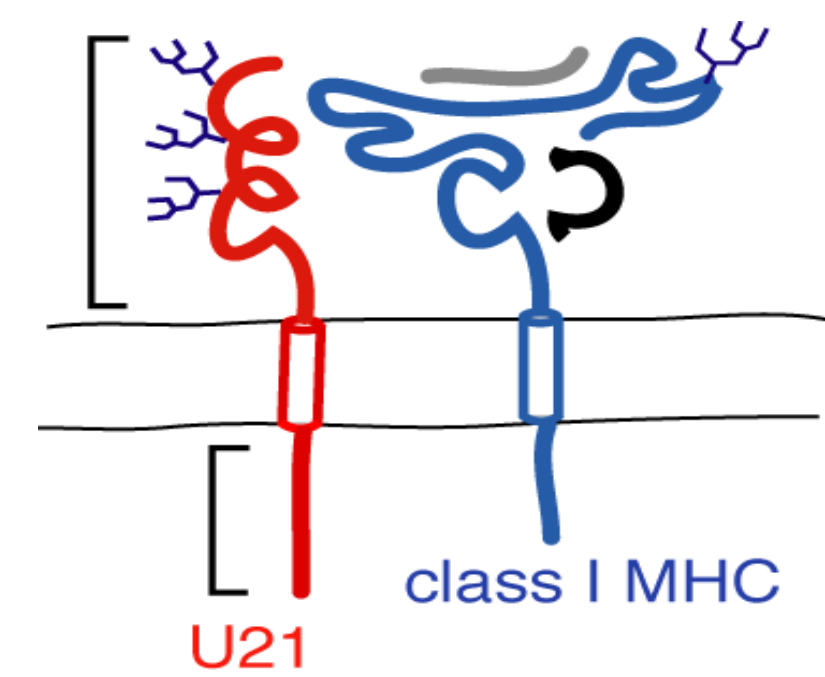
**Fig.1: Proper Assembly of Class 1 MHC.**

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2783374/figure/F1>



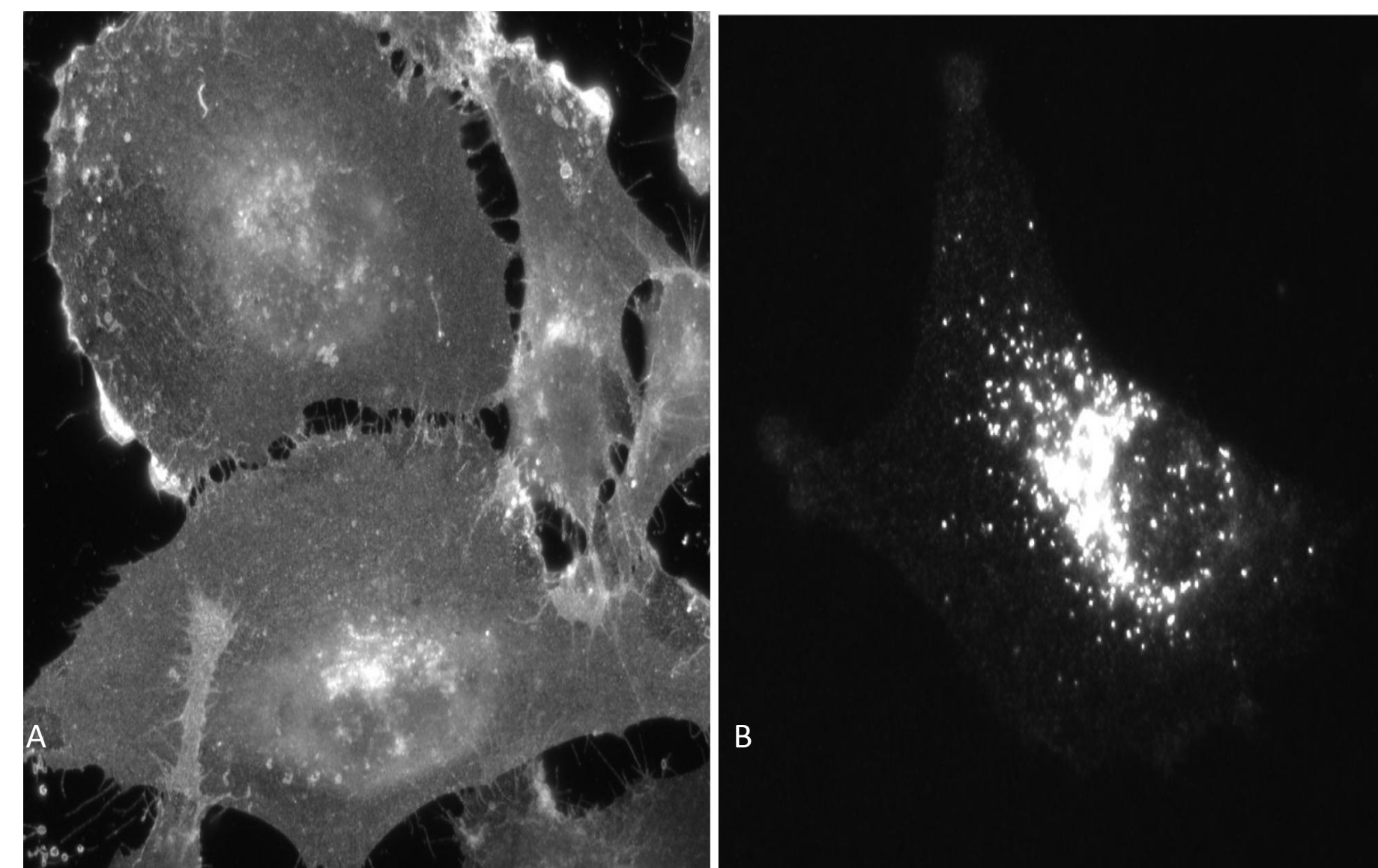
**Fig. 2. Structure Class 1 MHC loaded with peptide.**

**How Do We Know?** Dr. Hudson and associates are investigating the ability of HHV-7 to inhibit viral peptide presentation on Class I MHC molecules. These studies demonstrate that U21 (a viral protein associated with HHV-7) is able to effectively prevent the expression of Class I MHC on the surface of a cell. The exact mechanism of U21's inhibition of MHC expression is not fully understood. However, studies indicate that neither the cytoplasmic nor transmembrane domains interact with Class I MHC (Fig. 3). Additionally, no conformational change in the MHC peptide complex is observed.

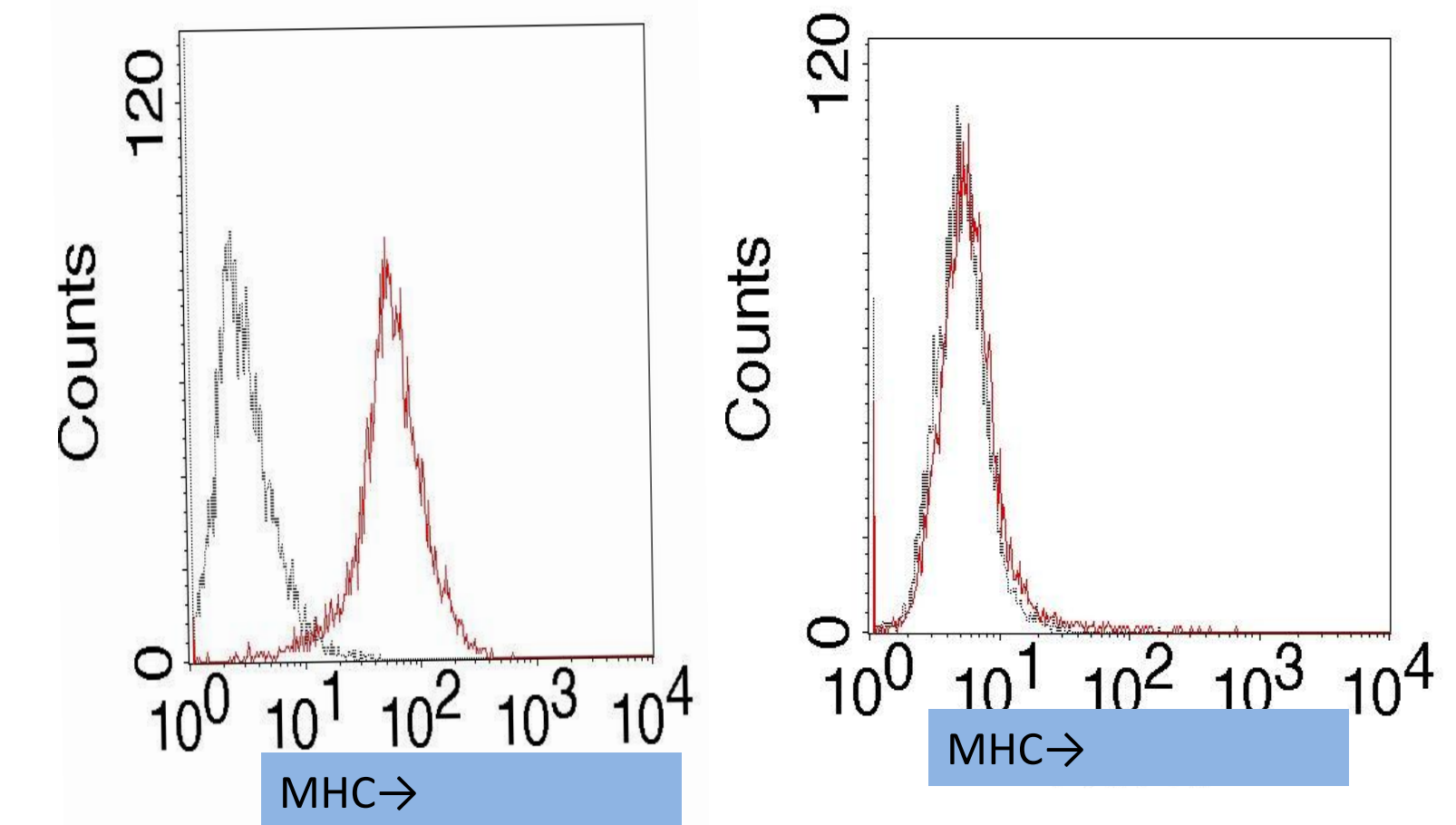


**Fig. 3. Proposed structure of U21 & Class 1 MHC**

Immunofluorescent studies indicate that U21 interaction with MHC diverts the molecule from the RER to the lysosomal compartments. This was demonstrated by labeling Class I MHC molecules with the antibody W6/32, and then adding a fluorescently-labeled secondary antibody. As seen in Figure 4, cells without U21 show a normal distribution of MHC on the cell surface and in the RER (Fig. 4a). However, U21-transfected cells show restriction of MHC in the lysosomal compartments and ER and no expression of MHC on the cell surface (Fig. 4b). The black peak of the Flow Cytometric Histogram of MHC without U21 (Fig. 5a) exhibits cells that do not express MHC on their surface, whereas the red peak shows cells that express MHC on the membrane. Fig. 5b displays no MHC on the cell surface because U21 is inhibiting MHC presentation.



**Fig. 4 . Immunofluorescent studies of Class 1 MHC location. (A) Normal expression of Class 1 MHC. (B) U21-expressing cells**



**Fig. 5 . Flow Cytometric Histograms of Class 1 MHC surface expression. (A) Class 1 MHC. (B) U21 Expressing Cells.**

**Story:** Class I MHC molecules are cell-surface proteins synthesized by ribosomes in the Rough Endoplasmic Reticulum (RER). HHV-7 proteins are synthesized on ribosomes located either in the cytoplasm or in the RER depending upon their function. Misfolded viral proteins are degraded by a proteasome, a macromolecular complex that digests aged and misfolded proteins marked by a small protein called ubiquitin. This generates Class I peptides that typically contain 8-10 amino acid residues and a hydrophobic carboxyl-terminal anchor. Viral peptides are transported into the RER by the transporter-associated with antigen processing (TAP) molecule. Within the RER, class I MHC  $\alpha$ -chains are properly folded with the aid of the chaperone molecule calnexin, and then associate with the  $\beta_2$ -microglobulin. The molecular chaperones Calreticulin, Tapasin, and ERp57 aid in forming a peptide-loading complex with an MHC molecule. Tapasin brings the MHC molecule into close proximity with TAP, while ERp57 stabilizes the interaction by forming a disulfide bond with Tapasin, and noncovalently associating with Calreticulin. The peptide loading complex stabilizes the MHC molecule until a peptide is loaded into the cleft. MHC with the bound peptide is then transported to the Golgi apparatus for vesicle packaging for release into the cytoplasm. Once it is released into the cytoplasm, the vesicle fuses with the cell membrane, and the MHC is presented on the cell surface and available for recognition by a T cell receptor.

**Future:** X-Ray Crystallography of U21 bound to Class I MHC would determine the structure of the protein, and aid in the discovery of U21's mechanism of diverting MHC to the lysosomal compartments from the Golgi. Since U21 down regulates classical and nonclassical Class I MHC, the mechanism of an alternative NK cell evasion must be discovered for proper understanding of HHV-7's ability to avoid NK destruction.

**Conclusion:** Class I MHC is a primary component in the cell-mediated immune system that is responsible for viral peptide presentation. HHV-7 is an elusive virus that avoids TCR and NK cell recognition by down regulating Class I MHC on a cell surface. By producing U21, HHV-7 controls down regulation by rerouting the Class I MHC molecules to the lysosomal compartments for degradation. The down regulation of Class I MHC allows the virus to remain latent in the body, undetected by the immune system.