Cell Biology from an Immune Perspective

In this lecture we will very briefly review some aspects of cell biology which are required as background knowledge in order to understand how the immune system works. These will include:

1. A brief overview of protein trafficking
2. Signal transduction
3. The cell cycle

Some of these issues will be treated in greater depth in later lectures.

Protein Trafficking/The Secretory Pathway:

From an immune perspective the secretory compartment and structures enclosed by vesicles are "seen" in different ways from proteins that reside in the cytosol or the nucleus. We will briefly review the secretory and endocytic pathways and discuss the biogenesis of membrane proteins. Some of the issues that will be discussed are summarized in Figures 1-3.

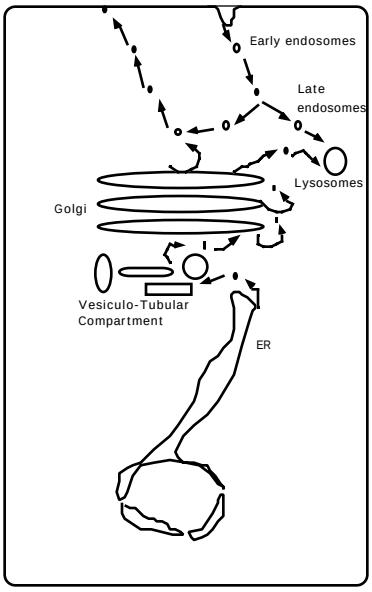


Figure 1. An overview of the secretory pathway

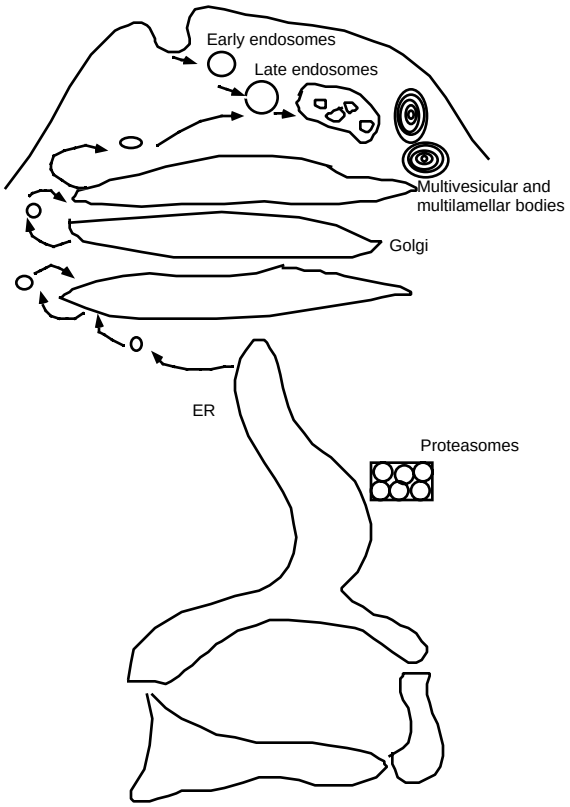


Figure 2. Protein degradation occurs mainly in lysosomes and proteasomes

Proteins that enter the cell from the environment are primarily degraded in lysosomes. Most cytosolic and nuclear proteins are degraded in organelles called proteasomes. Intriguingly these two sites of degradation are each functionally linked to distinct antigen presentation pathways, different kinds of MHC molecules and the activation of different categories of T cells.

Integral membrane proteins maybe inserted into the membrane in a number of ways, the two most common of these ways being considered in Figure 3.

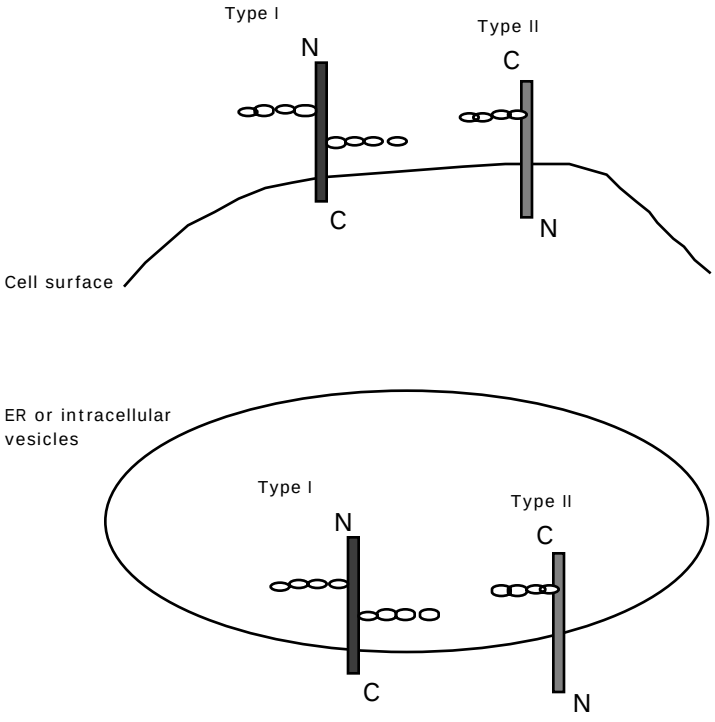


Figure 3. The orientation of Type I and Type II membrane proteins.

Antigen presentation pathways

Receptor mediated endocytosis will be discussed briefly in this lecture both in the context of the function of membrane bound immunoglobulins and in the context of antigen presentation pathways. Antigen presentation will be discussed in greater depth during the lecture by Hidde Ploegh and will be considered in broad outline only in this lecture.

T cells co-evolved with B cells. They have the ability to look “into” and destroy other host cells if the latter are infected. The basic design of T cell recognition depends on an intrinsic ability to ignore free or soluble antigens. T cell receptors can only recognize antigenic peptides that are appropriately exhibited on cell surfaces. The molecules that bring these peptides to the cell surface, and then exhibit them so that they may be scanned by passing T cells, are known as **Major Histocompatibility Complex** (MHC) proteins. These are the most polymorphic proteins known, encoded in most vertebrate species by a very large number of alleles.

The antigen receptor on T cells is made up of two transmembrane polypeptides of the immmunoglobulin superfamily, the T Cell receptor (TCR)) α and β chains. Receptor diversity is generated by the rearrangement, in developing thymocytes, of gene segments that encode these polypeptides. The variable domains of the TCR α and β chains contribute to the antigen binding site. TCRs recognize cell surface MHC molecules which are complexed with peptides of either exogenous or endogenous origin. It is now believed that all TCRs initially generated in the thymus are capable of recognizing MHC like shapes. We will return to this issue when we discuss why a relatively large proportion of T cells in any one individual have the potential to be alloreactive in later lectures on transplantation. The antigen recognizing TCR α and β chains associate with proteins of the CD3 complex, (CD3 γ, δ, ε, and ζ) which are required for the initiation of signaling from the antigen receptor.

MHC proteins evolved at the same time as B and T cells, to provide a mechanism for T cell receptors to focus exclusively on antigenic fragments that are held on the cell surface. Since T cell receptors recognize only MHC-peptide complexes, free viruses or viral antigens are totally ignored by T lymphocytes. Although the physiological function of MHC molecules relates to antigen recognition by T cells, they acquired their name because they were discovered in the context of transplant rejection. They were originally referred to as *Major* Histocompatibility Complex Antigens because they were identified as the most prominent antigens involved in experimental graft rejection. Elegant genetic studies, which helped create the field of immunogenetics, established that these antigens map to a single chromosome in the mouse, to a set of genes that came to be known as the H-2 locus. The corresponding locus in man is on chromosome 6 and is referred to as the **Human Leukocyte Antigen** or **HLA** locus. MHC molecules in man are called HLA molecules and the terms MHC and HLA will be used interchangeably. HLA molecules are found on leukocytes but

their expression is not restricted to white cells. MHC class I molecules are expressed on all nucleated cells and are recognized by T cell receptors on cytotoxic or $CD8^+$ T cells (Cytotoxic T Lymphocytes or CTLs). They generally present peptides derived from proteins synthesized “endogenously” in the cell expressing the MHC class I molecule of interest. MHC class II molecules are primarily expressed on B cells and other professional antigen presenting cells (mainly macrophages and dendritic cells). Their expression may be induced on endothelial and epithelial cells exposed to cytokines such as γ-interferon. MHC class II molecules generally present peptides derived from exogenous proteins which must be internalized by the antigen presenting cell. These proteins are broken down or “processed” into peptides intracellularly. MHC class II-peptide complexes are recognized by antigen receptors on helper or $CD4^+$ T cells.

The structure of MHC proteins

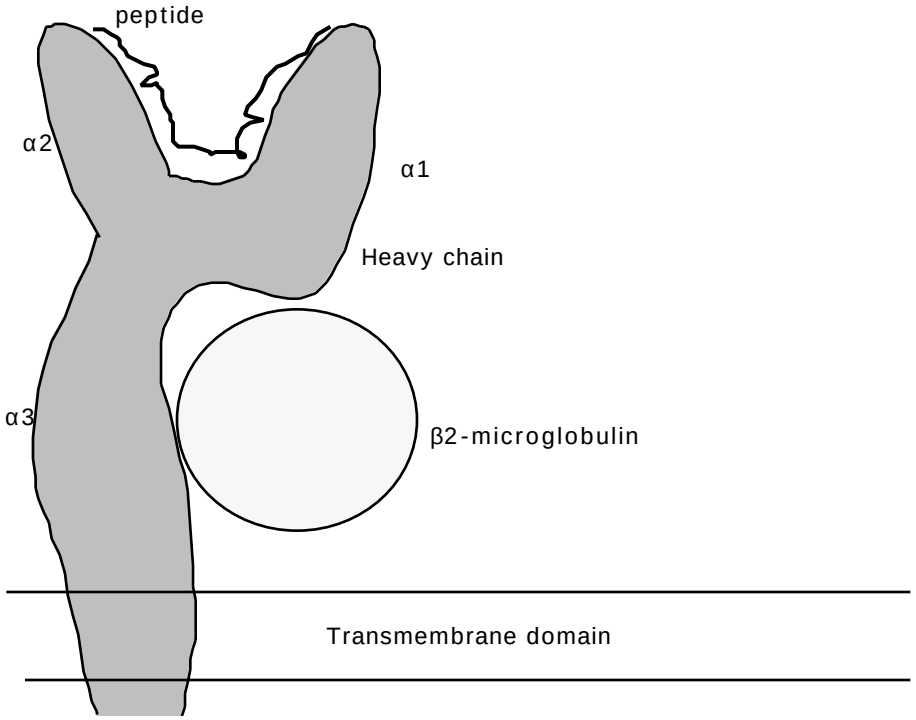
MHC molecules are cell surface glycoproteins which contain two membrane-proximal immunoglobulin like domains as well as two more distal specialized domains which together form a peptide binding groove embedded between two α-helical ridges. MHC class I molecules are made up of two polypeptide chains and a tightly associated peptide moiety (Figure 4). The MHC class I heavy chain is a transmembrane glycoprotein of about 40-45 kDa which associates tightly with a small non-anchored 12 kDa immunoglobulin superfamily protein known as β2 microglobulin. MHC class II molecules are heterodimers which consist of an α and a β chain, both of which are transmembrane glycoproteins (Figure 5).

Our current understanding of MHC function owes a lot to the knowledge of the structure of MHC molecules obtained by X-ray crystallography. Crystal structures are available for a range of HLA class I and class II molecules complexed with specific peptides and also, more recently for HLA class I -peptide -T cell receptor complexes. The MHC class I heavy chain is made up of three domains. The α1 and α2 domains combine to contribute to two α-helical ridges which surround a long, narrow, groove whose floor has a β-pleated structure. This groove is where specific peptides are snugly bound and presented to appropriate T cell receptors. The surface contains distinct crevices into which specific side-chains of a highly specific peptide may fit. Peptides that are presented by MHC class I molecules are usually 8 or 9 amino acids in length. For a particular MHC class I molecule, the specific crevices in the groove dictate that certain invariant amino acid side-chains must be present in peptides that bind specifically to

it. These residues on the peptide are referred to as anchor residues since they are critical for the tight binding of a set of peptides to individual class I
heavy chains

Figure 4. A schematic view of an MHC class I molecule.

The α3 domain of the MHC class I heavy chain has a typical immunoglobulin fold structure, consisting of a β sandwich where adjacent β sheets are held together by a disulfide bridge. This domain interacts with β2 microglobulin, a non-anchored protein



which also has a similar immunoglobulin domain like structure. The α3 domain is also involved in the interaction of MHC class-I molecules with the CD8 molecule on cytotoxic T cells.

The antigen binding groove on MHC class II molecules broadly resembles that found in MHC class I molecules. This groove is contributed to by the α1 domain of the α chain and the β1 domain of the β chain. The MHC class I groove is approximately 25A° long and is closed at both ends; it can therefore only accommodate relatively short peptides. The MHC class II groove is open-ended and can accommodate larger peptides. Non-polymorphic portions of the α2 and β2 immunoglobulin like domains contribute to the interaction with CD4 on T cells.

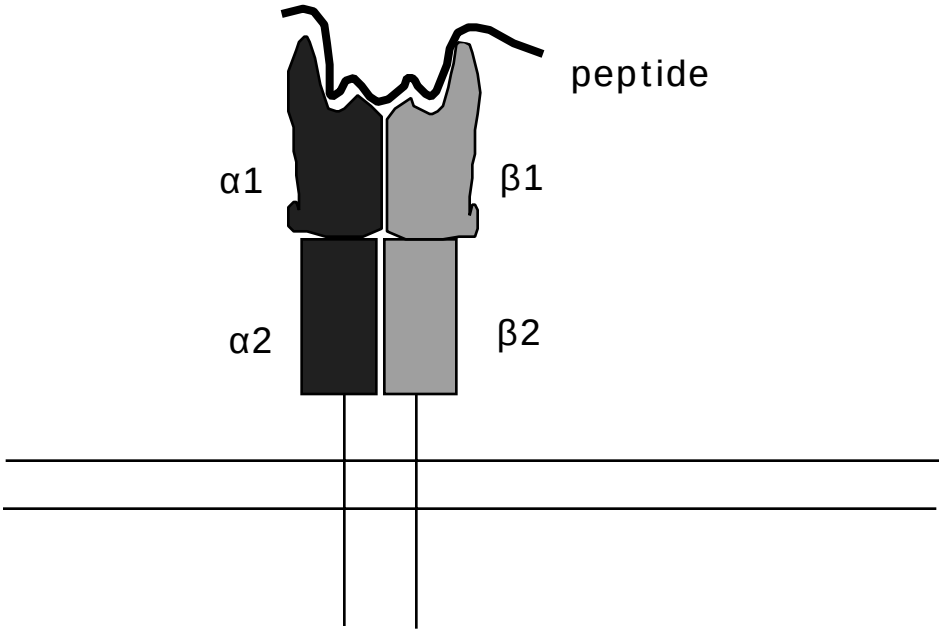


Figure 5. A schematic view of an MHC class II molecule

The MHC class I pathway

MHC class I molecules are designed to complete their folding and assembly processes in the endoplasmic reticulum (ER). Egress from the ER and subsequent transport to the cell surface requires that the complete trimolecular complex of heavy chain, β2 microglobulin, and peptide be properly assembled (Figure 6). The MHC class I heavy chain is translocated into the ER by a classic signal peptide dependent mechanism through the Sec 61 channel. Sec61 is an ER protein whose subunits form a channel through which polypeptide chains that contain signal peptides are translocated into the ER. The MHC class I heavy chain protein is anchored in the ER membrane and folds partially in conjunction with a resident transmembrane ER chaperone known as calnexin. In this compartment it associates with the β2 microglobulin component, which is also translocated into the lumen of the ER in a signal-peptide dependent manner. β2-microglobulin is not an integral membrane protein; it is initially translocated into the lumen of the ER, and can be secreted. The heavy chain-β2 microglobulin heterodimer remains associated in a complex with a protein known as tapaisin until it receives a peptide which is either 8 or 9 amino acids in length and which can fit into its specific groove. Tapaisin forms a bridge between MHC class I molecules and the TAP (Transporter associated with Antigen Processing) heterodimers which pump peptides into the ER from the cytosol. The TAP transporter is a

pump made up of two transmembrane ATPases, TAP1 and TAP2, that form a peptide-translocation channel in the ER membrane. TAP1 and TAP2 are encoded by genes that are a part of the MHC complex (discussed below).

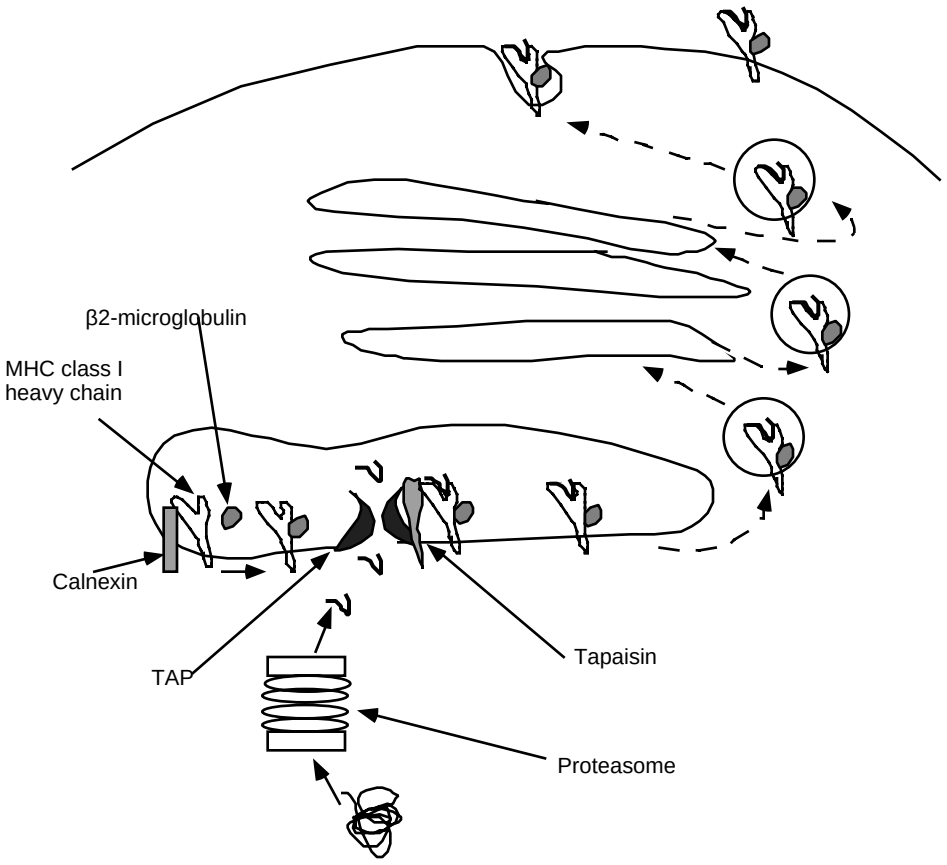


Figure 6. An outline of the MHC class I pathway.

Cellular proteins which lack signal peptides are generally destined for the cytosol and the nucleus. Most of these proteins are degraded in the cytosol in proteasomes. As a prelude to being degraded in proteasomes, many proteins are covalently tagged in the cell with tandem repeats of a peptide known as ubiquitin. Proteasomes are highly organized cylindrical protein-degradation machines made up of 4 circular segments of 7α, 7β, 7β, and 7α protein subunits respectively, which together make up the 20S proteasome. The 20S proteasome core contains a number of proteolytic activities. At either end of the core is a “cap” structure which combines with the 20S proteasome to generate the 26S proteasome. The cap contains proteins that can contribute to the unfolding and deubiquitination of target proteins. Three subunits of the proteasome, LMP-2, LMP-7, and MECL-1 may be induced by interferon-γ. LMP-2 and LMP-7, like TAP-1 and TAP-2, are

encoded within the class II region of the HLA complex on chromosome 6.

Although proteins that are targeted to proteasomes are primarily cytosolic or nuclear polypeptides, proteins retained within the ER as well as cleaved signal peptides may be translocated out of the ER lumen via the Sec 61 channel. Translocated proteins are ubiquitinated and targeted for proteasomal degradation. Proteins from the secretory pathway, including misfolded ER retained MHC molecules, may also therefore be targeted to the cytosol and to the MHC class I antigen presentation pathway.

The MHC class II pathway

Unlike the MHC class I pathway which is designed to eventually present cytosolically derived peptides to CD8 cytotoxic T cells, the MHC class II pathway is explicitly designed to present peptides derived from proteins that are sampled from the environment by professional antigen presenting cells. Even though MHC class II molecules are synthesized on ER bound ribosomes and assemble initially in the ER, they are designed NOT to sample peptides in the ER lumen itself (these peptides must be presented by MHC class I molecules). The α and β chains of MHC class II molecules assemble in the ER membrane and associate immediately with a third component known as the invariant chain (Ii). The invariant chain is a Type II transmembrane protein (in Type II proteins the carboxy-terminus protrudes into the lumen of the ER whereas in Type I proteins the N-terminus is exposed to the lumen). The C-terminus of the invariant chain folds into and occludes the antigen binding groove of the MHC class II molecule. Occupancy of the peptide-binding groove by the invariant chain prevents endogenous peptides that enter the ER from occupying the groove. This permits the efficient loading of MHC class II grooves with exogenously derived peptides in a post-Golgi compartment. The N terminus of Ii protrudes into the cytosol and provides targeting signals to direct $(\alpha\beta Ii)_3$ nonamers through the Golgi stacks to a specialized acidic late endosomal/lysosomal compartment known as the MIIc compartment. The invariant chain is proteolytically cleaved to yield a peptide called CLIP which remains tightly bound to the MHC class II groove.

Proteins from outside the cell are sampled and internalized by endocytic receptors on the antigen presenting cell. In the case of a B lymphocyte the antigen receptor functions as the endocytic receptor as shown in Figure 7. Internalized proteins are cleaved into peptides by proteases such as cathepsin-L and cathepsin-S. A distinct type of HLA molecule, a heterodimer known as HLA-DM,

performs a unique function in the MIIc compartment. This molecule serves as a peptide -exchanger, displacing CLIP from the MHC groove and permitting exogenously derived peptides to slide into the groove. MHC class II- peptide complexes are then transported to the cell surface. In general, peptides that bind the class II groove tend to be longer than those that bind to the corresponding groove in class I molecules - the average length of MHC class II binding peptides ranges from 14 to 18 amino acids, but since peptides can spill out of both ends of the non-occluded MHC class II groove they can even be considerably longer.

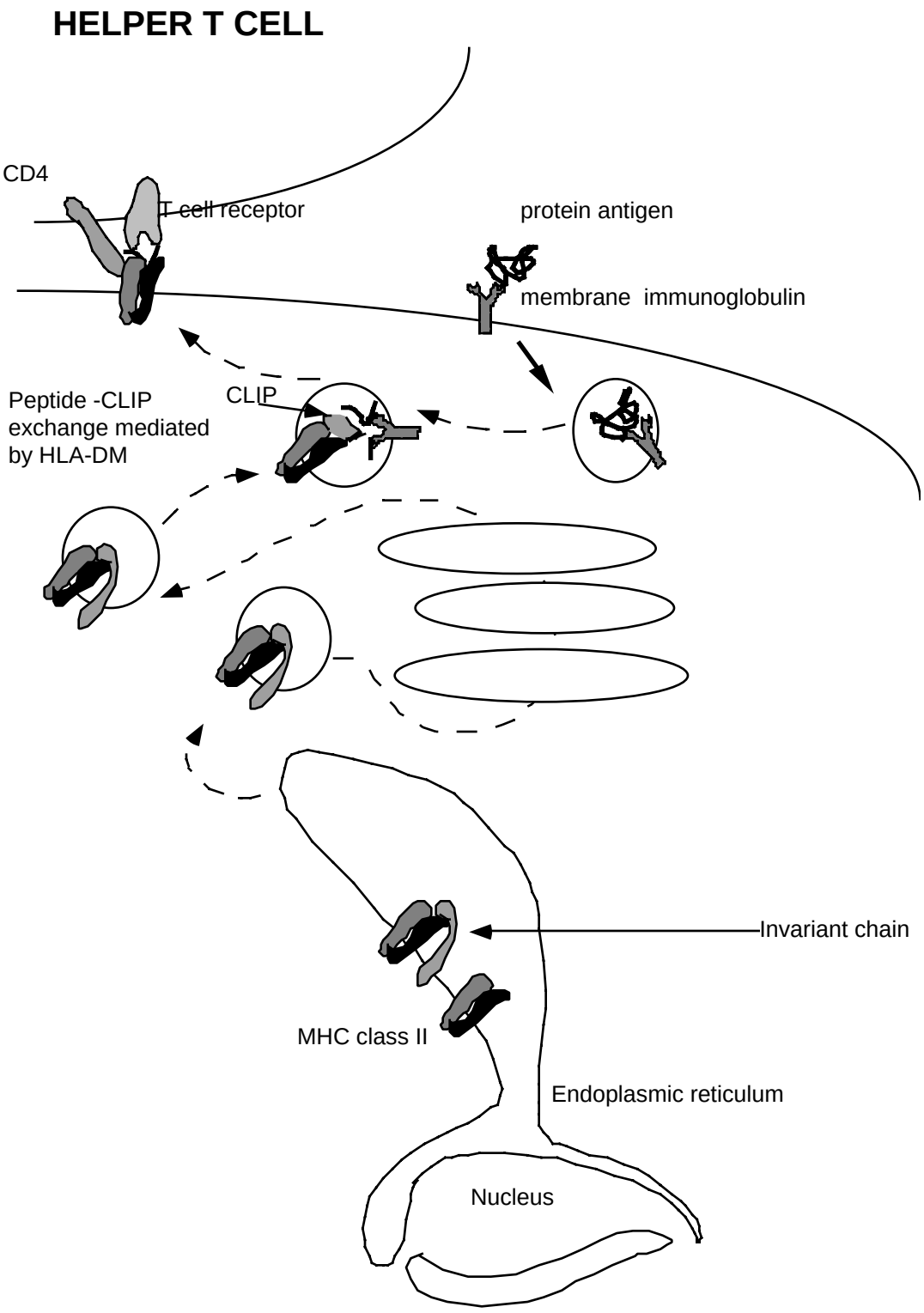


Figure 7. The MHC class II pathway.

An Overview of Signal Transduction

Every event in lymphocyte development is determined by signals. Most of these signals are delivered by surface receptors responding to external cues. Some developmental transitions may be driven by receptors which do not require extracellular ligands but which generate constitutive or “tonic” signals to drive cells past specific checkpoints. Most developmental signals lead either to the post-translational modification of key cytoplasmic proteins or the regulation of gene expression, or both. The initiation of a signaling pathway might lead to one or more of a number of events. These include the induction of survival factors, the initiation of proliferation, the activation of a differentiation program, or, in certain circumstances, the triggering of cell cycle arrest or of cell death.

A brief overview of receptors that initiate signal transduction

Signal transduction is usually initiated at the cell surface by the binding of extracellular ligands to integral membrane proteins which function as receptors (Figure 8). A special category of membrane permeable ligands may initiate signal transduction, not at the cell surface, but by directly interacting with nuclear receptors. While the majority of receptors of direct importance in lymphocyte development are cell surface receptors, nuclear receptors also participate in the process. Some signals may also be initiated and transduced purely from intracellular locations. Examples of this latter kind of signaling include the checkpoint pathways that are transduced in cells following DNA damage or when chromosomes fail to segregate appropriately. Most of the issues that we will discuss in this chapter reflect the initiation of signals from the cell surface.

Signal transduction pathways generally consist of a number of sequential steps which may culminate in nuclear or non-nuclear events. The existence of multiple steps in a pathway offers the opportunity for multiple branch-points and therefore for a single initial molecular event to lead to the functional modification or activation of a large number of targets. The existence of multiple intermediate steps also allows for signal amplification, although this could well be achieved without the need for many steps in the pathway.

Some signaling pathways may be initiated from cell surface receptors which may themselves be protein kinases or which may be physically linked to protein kinases. Protein kinases in vertebrates either phosphorylate proteins on tyrosine residues (protein tyrosine kinases) or on serine or threonine residues (serine/thronine kinases). In the immune system, a large number of receptors physically associate with cytosolic protein tyrosine

kinases, some of which snuggle up to the inner face of the plasma membrane. Crosslinking of receptors that belong to this category (which include antigen receptors and the majority of cytokine receptors) leads to the activation of the linked tyrosine kinase and the subsequent initiation of signaling pathways. A number of receptors of importance in the immune system are not known to be directly linked to tyrosine kinases.

However ligation of these receptors leads to the activation of downstream pathways because the cytoplasmic tails of these receptors are induced to recruit critical signaling effectors that mediate their function without necessarily inducing a specific phosphorylation or de-phosphorylation event. Although receptors such as the CD40 molecule, or those for cytokines such as TNF and IL-1, may not be directly associated with protein kinases, nonetheless they initiate a number of signaling pathways which depend on the activation of protein kinases.

A distinct category of receptors, often referred to as serpentine receptors, is made up of proteins which contain seven transmembrane domains and which are coupled to heterotrimeric G-proteins (which bind GTP and GDP). While receptors of this category exist in virtually every cell, from the viewpoint of lymphocyte development a particularly important group of serpentine receptors are those that mediate responses to chemokines.

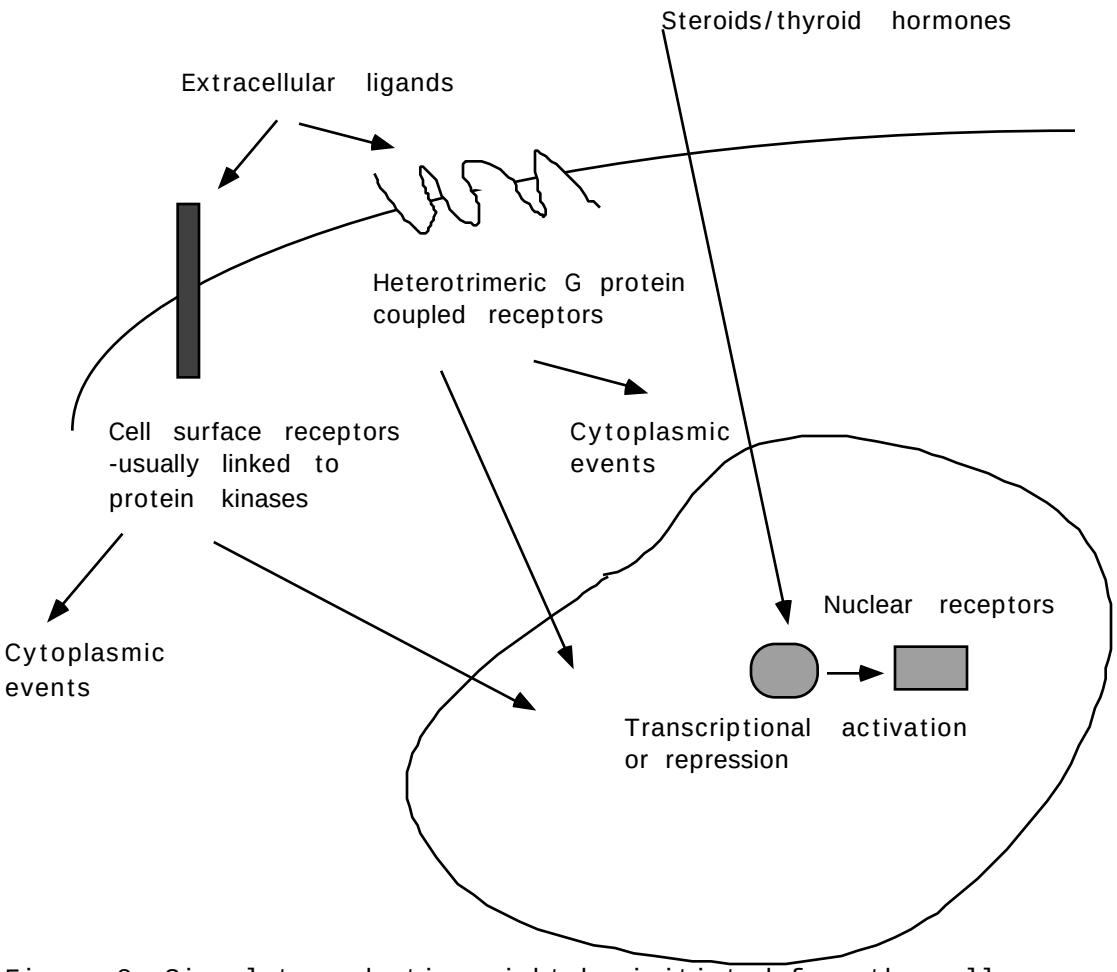


Figure 8. Signal transduction might be initiated from the cell surface by extracellular ligands or by membrane-permeable ligands which interact with nuclear receptors.

Nuclear receptors are basically transcription factors which contain a ligand binding site for membrane permeable mediators such as glucocorticoids or vitamin D. These ligands bind to the nuclear receptors and this leads to a conformational change which permits this protein to function as a specific transcriptional activator or repressor. Glucocorticoids may participate in T cell development.

Signaling via cell surface receptors may be transduced in many locations within the cell. Signal transduction pathways may target cytosolic proteins, they may lead to the post-translational modification of membrane proteins which maye be other signaling receptors or ion-channels, and they might also influence nuclear function, generally by regulating gene expression.

Kinases and phosphatases involved in cellular signaling

Protein phosphorylation regulates almost every aspect of cellular function. In lymphocytes, proteins may either be phosphorylated on tyrosine residues by tyrosine kinases, or on serine or threonine residues by serine/threonine kinases. Certain kinases are capable of phosphorylating both serine/threonine residues as well as tyrosine residues, and are known as dual-specificity kinases. These phosphate residues may be removed in turn by phosphatases which may be tyrosine phosphatases, or serine/threonine phosphatases, or dual-specificity phosphatases. Regulation of cellular events may therefore often be achieved by modulating the activity either of a specific protein kinase or by influencing the activity of a protein phosphatase.

Although most of the phosphorylation events that we consider involve proteins as substrates, it should be kept in mind that kinases and phosphatases that modify lipids are also critical players in signaling.

The regulation of G1 progression by extracellular signals

A resting or G0 cell may be induced to move out of this quiescent stage by signals that increase the overall efficiency of transcription and translation in the cell. We have just considered how translation may be enhanced by a number of signaling pathways. External signals during G1 may drive a cell to progress further in the cell cycle. In G1 the cell needs to be driven into the S phase of the cell cycle during which DNA synthesis occurs as a prelude to mitosis (Figure 9).

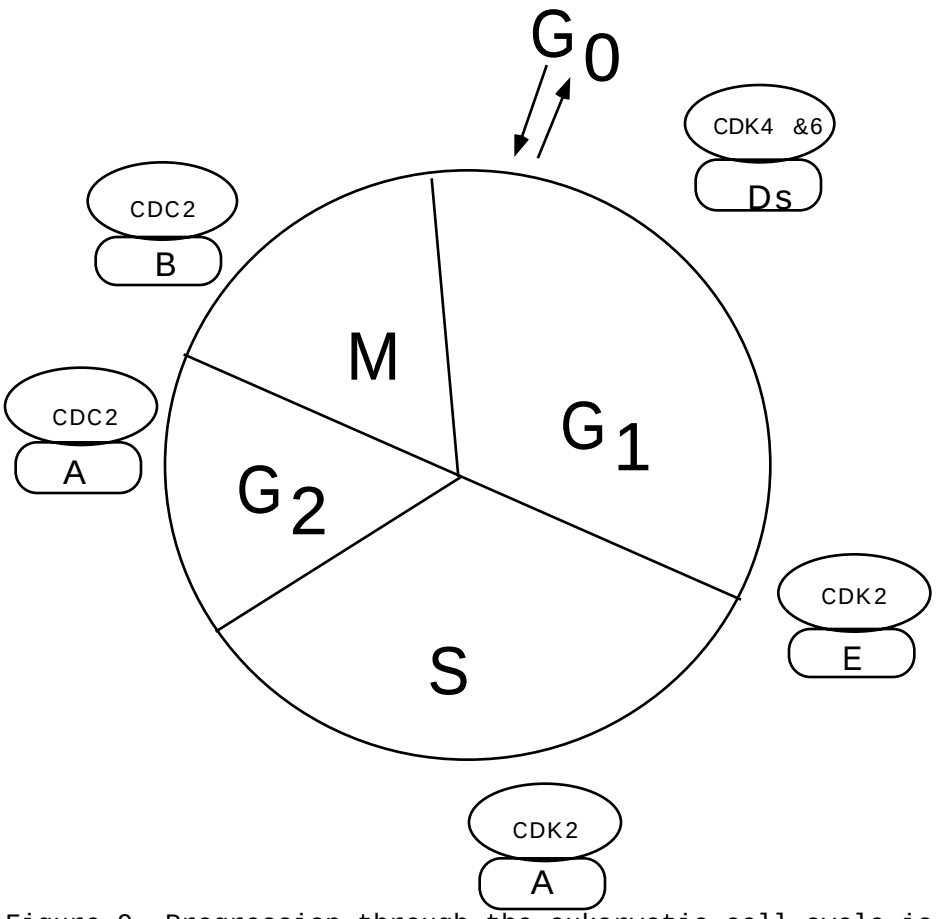


Figure 9. Progression through the eukaryotic cell cycle is regulated by cyclin dependent kinases, which are active only in complex with a cyclin partner

External signals that drive proliferation do so primarily by contributing to the activation of specific Cyclin Dependent Kinases (CDKs), that are critical in the G1 phase of the cell cycle. CDKs are proline directed serine-threonine kinases and,as their name implies, are active only when they are complexed with cyclins. Cyclins are small proteins which are non-catalytic regulatory subunits of CDKs but which are degraded in a cyclical fashion during the cell cycle. There are a number of different types of cyclins which are required at distinct stages of the cell cycle. The cyclins which are critical in the early to mid G1 phase are D-type cyclins. In lymphocytes, cyclin D2 and cyclin D3 are the members of this class which are induced in G1and which associate with and activate CDK4 and CDK6. Late in G1 the cell cycle is driven by CDK2/cyclin E complexes and CDK2/cyclin A complexes help drive cells through the S phase. Mitosis depends on the catalytic activity of CDC2 containing complexes.

Summary

- Proteins that contain hydrophobic N-terminal or internal signal or leader peptides are co-translationally or post-translationally translocated into the endoplasmic reticulum. These proteins may either reside within the secretory pathway, may be destined for lysosomes or may be transported to the cell surface by vesicular transport.
- The Major Histocompatibility Complex Antigens were initially identified as prominent antigens involved in graft rejection. The murine MHC locus is called the H-2 complex. The syntenic human chromosomal region is called the Human Leukocyte Antigen (HLA) locus.
- T-cell recognition of antigen requires that antigenic peptides be displayed within the cleft of a self-MHC molecule at the cell surface. This requirement is called self-MHC restriction. $CD4^+$ helper T cells are class II MHC restricted, and $CD8^+$ cytotoxic T cells are class I MHC restricted.
- MHC Class I proteins are expressed on all nucleated cells and present antigenic peptides derived endogenously within the cell. Endogenous antigens are degraded into peptides within the cytosol by the proteasome (containing several peptidase activities). The resulting antigenic peptides are transported into the lumen of the rough endoplasmic reticulum (RER) by TAP, an ATP-dependent peptide translocator. MHC Class I proteins consist of two subunits: the membrane-anchored heavy chain protein and the soluble β2-microglobulin (β2M) protein. The protein tapaisin forms a bridge between MHC I and TAP and aids in the formation of the MHC-peptide complex. Once peptide is bound, the MHC complex is stable and moves from the RER through the Golgi out to the plasma membrane.

- MHC Class II proteins are expressed by B cells and other professional antigen presenting cells (i.e., macrophages and dendritic cells) and present exogenous peptides. Exogenous antigens, internalized by phagocytosis or endocytosis, are degraded by various hydrolytic enzymes within lysosomes. In the ER, newly synthesized and translocated MHC Class II molecules associate with the invariant chain, which blocks binding of cellular peptides. The MHC II molecules then move through the Golgi into endocytic vesicles, where the invariant chain is degraded by proteases, leaving the CLIP fragment bound to the MHC. As the MHC-CLIP moves to lower-pH compartments, antigenic peptides displace CLIP. The MHC-peptide complex then moves to the plasma membrane.

Objectives/Study questions

1. What is the secretory pathway? What is a signal or a leader peptide?
2. What are the major sites of protein degradation in the cell?
3. What is receptor mediated endocytosis?
4. Trace the path of a newly synthesized immunoglobulin heavy chain protein of the membrane form, from the initiation of translation to the cell surface. Is the pathway by which this protein makes its way to the cell surface the same route taken by an MHC class II α or β chain? Explain.

Questions about MHC class I and class II molecules and antigen presentation are provided later in the camel (see 9/21/00).