COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH UNIVERSITY GRANTS COMMISSION

LIFE SCIENCE CODE: 03

IMMUNOLOGY

The immune system is a host defence system which protects an organism against diseases. The English word immunity comes from a Latin term *immunis* meaning "exempt". Primarily immune system is divided into two parts → innate immunity and adaptive immunity.

In early research in immunology scientist though that there are two components that helps to maintain the cell immunity. These are soluble proteins in body fluid (immunoglobulins in serum) and the cells of body fluid (leukocytes and lymphocytes). According those scientists divided the whole immune system as:

1. Humoral immunity: It is humoral component (immunoglobulin or antibodies) mediated immunity. It is called the humoral immunity because the antibodies were contained in body fluids (in previous year body fluid known as body humour). it is further divided into passive and active immunity.

   Passive immunity: transfer of antibodies or immune cell from an immune to a non-immune individual. E.g. treatment of snake or scorpion bite victims by immune serum which contains antibodies against snake or scorpion venom. It is a very quick and short-lived process.

   Active immunity: it is one's own immunity. It may hamper because of vaccination and natural infection. It is a renewable long-lived protection.

2. Cell mediated immunity: it is mediated by specific cells. In 1950s lymphocytes were first identified which has a role in both humoral and cell mediated immune system. Hence these two systems are interwinds and important for complete immune response.

Innate immunity: innate immunity is work as a first line defence system against various pathogen. It includes physical and chemical barriers (describe later). It includes pattern recognition receptors (PRR), complements that initiate a cascade of levelling and destruction procedure.

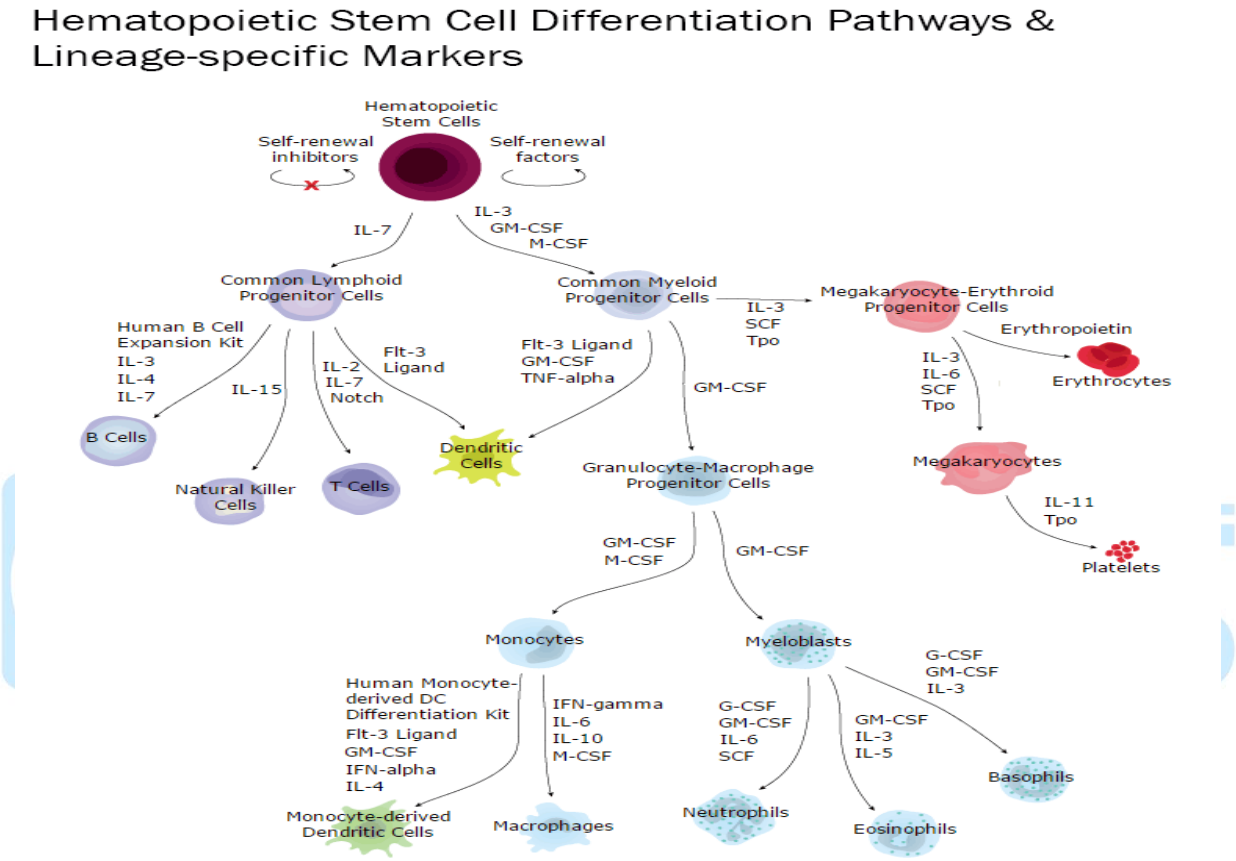
Adaptive immunity: the second form of immunity is the adaptive immunity. It relies on B and T lymphocytes. It takes long time and the components of adaptive immune system is very specific.

Innate and adaptive immune system are not independent. They work together. The communication is held by both cell-cell contact or by soluble messengers e.g. cytokines.

Cells of immune system

All the cells of our body are formed from stem cells which have ability to self-renew and form diverse cell types during embryogenesis. These embryonic stem cells are pluripotent. From pluripotent stem cell progenitor stem cell are formed which is specific for particular tissue. Haematopoietic stem cells (HSCs) are stem cell from which all types of mature blood cells (erythrocytes, granulocytes, macrophage, dendritic cell, lymphocytes) are formed by the

process of haematopoiesis. HSCs makes two broad linages a common myeloid-erythroid progenitor from which all erythrocytes thrombocytes, leucocytes are formed and common lymphoid progenitor form which B lymphocytes, T lymphocytes, NK cells are formed. GATA2, a transcriptional factor, is required for development of all hematopoietic lineages. Bim-1 another transcriptional factor required for self-renewal capacity of HSCs. Ikaros and notch are both families of transcription regulators are required for only lymphoid development.



Cells of common myeloid-erythriod progenitor (components of innete immunity): the common myeloid progenitor contains erythroid cell(RBC), megakaryosites and myeloid cell (granulocytes, macrophage, monocytes, dendrituc cell).

- Granulocytes: granulocythes contains nutrophiles, besophiles, mast cell and eosinophiles. They are called granulocytes because their cytoplasm contains granules which is full of various proteins helps in damage of pathogen directly and regulate the trafficking and activity of other white blood cells. Neutrophiles are most abundent among all WBC. It constitute the 50%-60% of circulating leucocytes. Amount of circulating neutrophiles are increased gradually at the site of infection which is called leukocytosis. It has phagocytic cell it can engulf the bacteria by phagocytosis. It also

release chemokines which helps in accumulation of other neutrophiles (initiation of inflamatory response).neutrophiles are dominant first responders to infection.

Basophiles are nonphagocytes granulocytes. They are very rare in circulation (<1%). Basophiles release their granule which contain proteins mainly histamines in response to binding of circulatory antibodies. Basophiles are important for response to parasite and histamines are responsible for allergic symptoms. Basophiles may release cytokines.

Mast cell are presents in a low concentration (<1%) in blood. It does not mature when it release from bone marrow into the blood. It only mature when it leaves the blood. It founds in respiratory, urogenital, digestive tract, skin, connective tissue, mucosal epithelial tissue. They also have granules which contain histamins that play a role in allaergic reaction.

Eosinophiles present in the bloos at low concentration (1%-3%). Neutrophiles are phagocytic cell and contain granule. They form a cluster around the multicellular parasites and release their granules which destroy the parasites. Eosinophiles also can release cytokines.

- Myeloid antigen presenting cell: myeloid antigen presenting cells (APCs) contain monocytes, macrophages, and dendritic cell. They form a cellular bridge between adaptive and innate immunity. APCs ingest the pathogen via phagocytosis and digest phathogenic protein into peptides and APCs present those peptide antigens on their membrane surface and ultimately which leads to the activation og t lymphocytes. This process is called antigen presentation.

  The concentration of the monocytes in the blood is about 5%-10% of WBC. After releasing from the bone marrow it classified into two broad catagories. Inflamatory monocytes which have role in immune system as a effective phagocytes. They act as antigen presenting cell and helps in clearence of pathgens. Another one is patrolling monocytes who provide a reservoir for tissu resident monocytes in absence of infection. Monocytes migrates into the tissue in response to the infection and differentiate into macrophage. Some macrophage regulates their repair and regenaration. Others helps in innate immune system. Microphage can bind to a anigen more readily when the antigen is coated by an antibody. Thus antibody ia act as opsonin, a molecules that binds an antigen marking it for recognition by immune cells. And the modification of a particular antigen by antibody is called opsonization.

Names of Macrophages According to Tissue Locations

Location	Name
Connective tissue	Histiocyte
Serous cavity	Peritoneal macrophage
Liver	Kupffer cell
Bone tissue	Osteoclast
Lung	Alveolar macrophage (dust cell)
Nervous system	Microglial cell
Spleen	Sinusoidal lining cell
Skin	Langerhans cell
Inflamed tissue	Infiltrating macrophage

Dendritic cells are important immune cells. They are covered with membranous extention which is resembles the dendrites of nerve cells so these are called dendritic cell. Dendritic cells can form from both myeloid and lumphoid progenitors. They capture the antigen in one location and present the antigen in another location. Dendritic cell and folecular dendritic are fully different in nature. Folecular dendritic cells are not act as APCs. It found in lymph follicles which have a important role in B cell maturation.

- Erythroid cell: erythrocytes or red bloos cells (RBC) are formed from common myeloid and erythroid progenitor. RBC contain high concentration of hemoglobin. RBC helps in transport of oxygen in various tissue. It also release signals that induced innate immunity. All mammalian RBC does not contain nucleus and almost all non mammalian vertibrates retain nucleus.
- Megakaryocytes: megakaryocytes are the large myeloid cells that form thousand of platelets which helps in formation of blood clot.

Cells of lymphoid progenitor (components of aduptive immunity except NK cell):

The cells of common lymphoid progenior includes lymphocytes. They are broadly classified into three types: B lymphocytes (B cells), T lymphocytes (T cells) and natural killer cells (NK cells). They represent 20%- 40% of circulating white blood cell and the 99% of cells in lymph. The surface protein of the innune cells are refered as cluster of differentiation or designation (CD) nomenclature.

B lymphocytes are produce in bone marrow and also matures in bone marrow. The name B lymphocytes came form its site of maturation in bursa of Febricius in birds which is similar to bone marrow in human, mice and other mammals. B cell display B cell receptor called BCR which is a membrane bound immunoglobin (antibody) that binda to antigen. B cells have surface antibody. B cell can increse their binding ability to antigen by the process of somatic hypermutation and they can express different functional classes of antibody by the prpcess of class switching. Activated B cell further differentiate into the plasma cell which lose the

expresion of surface antibody and highly specialized for secretory antibody from a few hundred to more then a thousand molecule of antibody per second.

T lymphocytes are formed from bone marrow and mature in thymus. The name T lymphocytes came from its site of maturation. T lymphocyte doesn't contain surface antibody. T cell also contain T cell receptor (TCR). T cell receptors only recgnize the processed pieces of antigen bound to the major histocompatibility complex (MHC) molecule, a cell membrane protein which expressed in all nucleated cell of vertebrate species (class I MHC molecule) or expressed by professional antigen presenting cells and few other type of cells during inflammation (class II MHC molecule).

T cells are mainly two types – **T helper (T_H) cell** and **T cytotoxic (T_C) cell.** Genenrally T_H cell displaying CD4 gycoprotine s on their surface and recognise antigen in complex with MHC class II. CD4+ t cell browse the surface of antigen presenting cells with their T cell rceptors. When they intract with MHC peptide complex, they can be activated and proliferate and differentiate into a variety of effector T cell subset e,g T_H1, T_H2 etc. Another type of CD4+ cell is regulatory t cell (T_{REG}). Regulatory T cell has a capacity to inhibit an immune response. These cell are devided into two types – natural T_{REG} and induced T_{REG}. These cells are identified by the expression of CD4 and CD25 on their surface and internal transcription factor FoxP3.

T_C cell displaying CD8 glycoprotein on their surface and recognize the antigen in complex with MHC class 1 molecule. These cells also browse the surface of antigen presentin cell with their T cell receptors. When they interect with MHC peptide complex, they activated and proliferate and differentiate into an effector cell called cytotoxic T cell (CTL).

Natural killar cells are lymphoid cell and considered as a part of innet immun system. NK cells does not express antigen specific receptor. They express a specific marker in their surface known as NK1.1and they also have cytotoxic granules. They also called large granular lymphocytes. NK cells work in a different way. They recognise the absence of MHC I molecule which is presennt almost all normal cell but it is down regulated in respose of some viral infection or tumor cells. They recognise it by their specific receptor for MHC I molecule and and release cytotoxic granules and killed the effected cell.

NKT cells are another type of lymphocytes cell. They shares a common fearures with both NK cell and T cell. Like T cell they laso have TCRs and often express CD4. Like NK cell they have antibody receptor and other receptor assotiated with NK cells. When NKT cell activates they release cytotoxic granules to kill the target cell but also release cytokines. They appear tobe involve in human asthma as well as inhibit the cancer. Understading the exact work of NKT cells is under research priority.

LYMPHOID ORGANS — PRIMARY AND SECONDARY LYMPHOID ORGANS :

Lymphoid organs are those that have microenvironment that support the maturation proliferetation and differentiation of T and B lymphocytes.

There are two types of **primary lymphoid** organ in which T and B lymphocytes are synthesized and matured. These are bone marrow and thymus.

Bone marrow is a primary lymphoid organ that supports the synthesis and maturation of B lymphocytes although it also supports in self renual and differentiation of hematopoitic stem cells into mature blood cell. After the maturation of B cell it transported to secondary lymphoid organ through circulating blood. In bird B cell matures in bursa of fabricius.

Thymus is an important site for T cell maturation. Thymocytes (immature T cells) are passed through defined developmental stages to form mature t cell in thymus. Thymus is devided into two parts the cortex which is densely populated with DP (double positive) immature thymocytes and the medulla which is sparsely populated with single positive (SP) mature thymocytes. There is a major region named corticomedullary junction (CMJ) by which cell enters and exit to the blood stream. In subcapsular cortex (the region between cortex and thymic capsule) is a site of proliferation of the youngest (double negetave;DN) thymocytes.

After synthesis and maturation of B and T lymphocytes in primary lymphoid organ they transported into the **secondary lymphoid organs** where they encounter antigen and initiate an immune response.

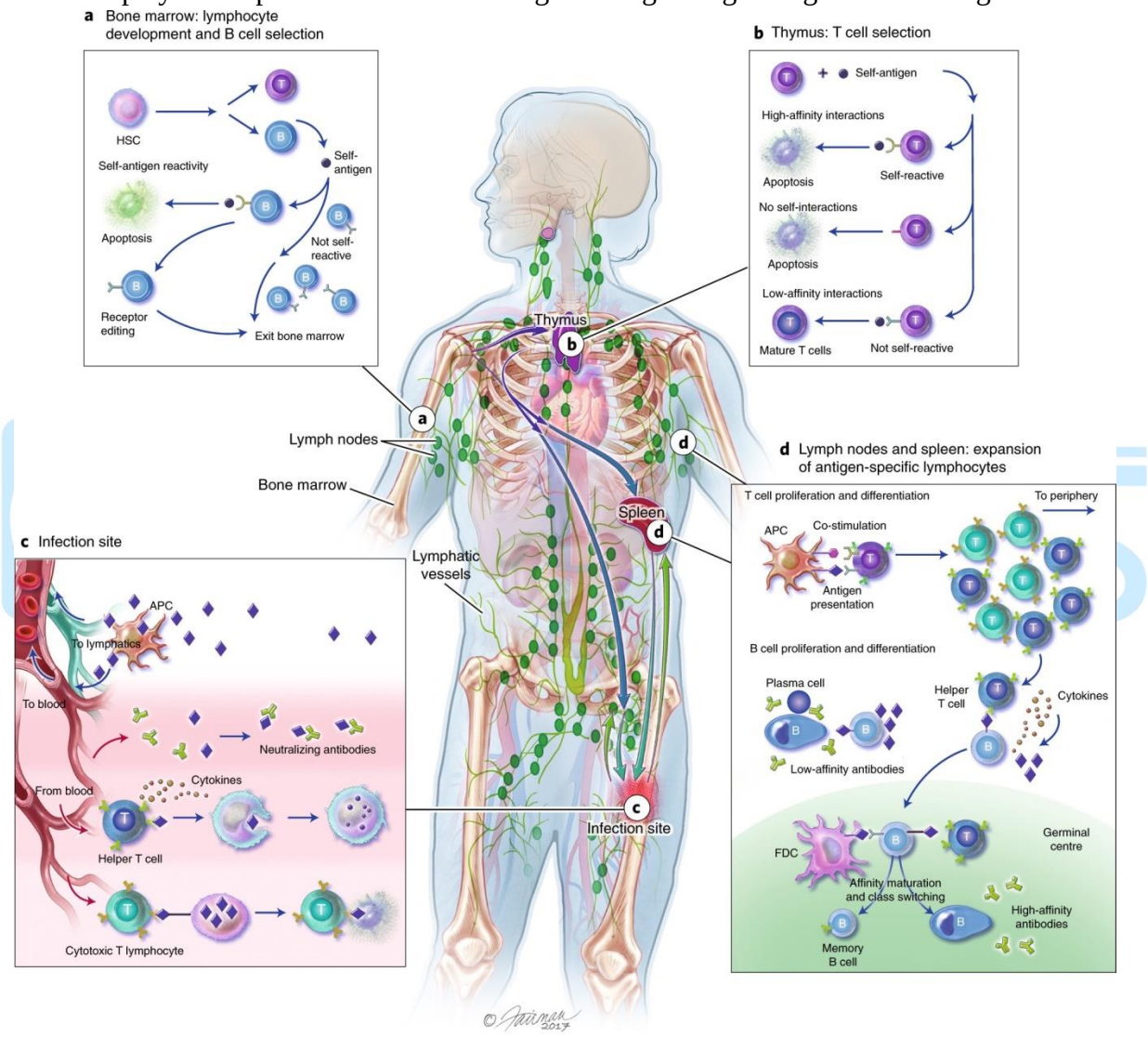
The major secondary lymphoid organs are lymph nodes, spleen and mucosa assotiated lymphoid tissue (MALT).

Lymph nodes are the most specialized secondary lyumphoid organ. Lymph nodes are encapsulated and bean shaped organs which are the first organized lymphoid structure to encounter the antigen that enter into the tissue spaces. Lymph nodes have three parts: cortex which contains mostly B cell, macrophages, and dendritic cell arranged in a follicle. Paracortex which contains mostly T cells, dendritiuc cell that migrated from tissue to node. Medulla, the innermost layerfrom ehere lymphocytes exit the lymph node. Lymph nodes have germinal centers which facilatrs the generation of B cell with increased receptor affinity. Unactivted B cell returns into the follicles and formed germinal centre where secendary follicle are present. The follicles with out germinal centers called primary follicle.

Spleen is a large ovoid secondery lymphoid organ which present high in the left side of abdominal cavity. Spleen is specialized for systemic infection that is trapping the blood born antigen and helps in filtering the blood. Spleen has some specialized region called red pulp and white pulp which are seperated by marginal zone. Red pulp contains red blood cells , macrophages, and some lymphocytes. In red pulp ols and defective red blood cells are destroyed and removed. White pulp contains T lymphocytes as well as B follicles. Marginal zone (border of white pulp) contains B cell and specialized macrophoges which shoes the first line of defence.

Mucosal membrane, that lines the respiratory, digestive, urogenital system and skin, are the major site of entry of most pathogens. A group of lymphoid tissue called **mucosa asociated**

lymphoid tisue (MALT) protects these system from pathogens. According to the area of the mucus MALTs have some specific name i.e. bronchus associated lymphoid tissue (BALT) in rspiratory system, gut associated lymphoid tissue (GALT) in intestine. The outer mucoal epithelial layer contains intraepithelial lymphocytes mostly T cell. The layer under the epithelial layer called lamina propia contains large number of B cell, plasma cell, activates T cell and macrophages. MALT have some special charecteristics that the epithelial cell in mucus embrane plays an importanr role in delivering of foreign antigens e.g. M cells in digestive tract.



Skin also plays an important non specific barrier against pathogens. Outer layer of skin contain keratinocytes a specialized epithelial cell which produce cytokins and induce local inflamatory reaction. Skin also have Langerhans cell, a skin resident dendritic cell , acts as potent activator of natïve T cells. Skin also have intra epidermmal lymphocytes which is mainly T cell, scattered dendritic cell, monocyte, macrophage as well as hematopoietic stem cells.

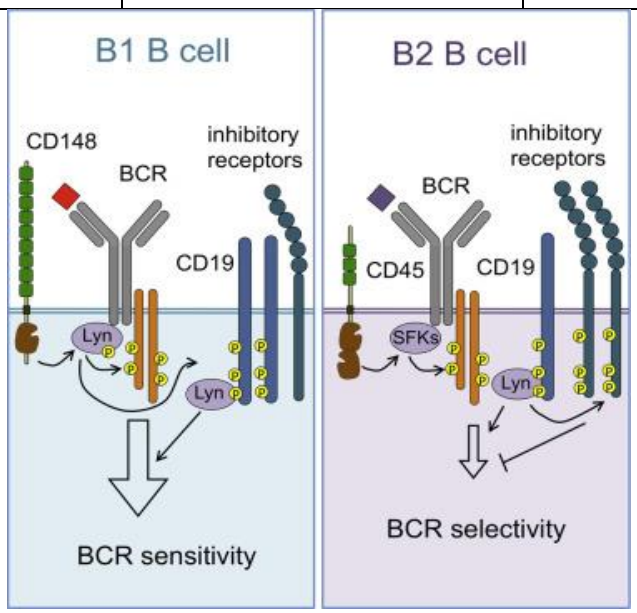
B CELL — DEVELOPMENT, ACTIVATION, DIFFERENTIATION AND MEMORY GENERATION AND B CELL RECEPTOR

B cell development

In adults B cell produced in bone marrow. But in embryo hematopoisis held in the yolk sac, placenta, as well as aorta-gonad –metanephros (AGM) region and the fetal liver. In fetal liver B cell progenitors form B-1 B cells which migraters in to the pleural and peritonial cavities. B-1 B cells are remain self renewing throughout the life of the animal. B-2 B cells are conventional B cell which is the major group of B cell in human. B-2 B cell also known as follicular B cell. Mature B-2 B cell reirculte between blood and lymphoid organ. They s can be found in the B cell follicles of the lymph node and spleen.

Comparison between properties of B-1 cells and B-2 cells:

Attribute	B-2 B cell	B-1 B cell
Primary location	Secondary lymphoid organs	Peritonial and pleural cavities
Source of new B cell	From precursors in bone marrow	Self renewing
Requirements of T cell help	yes	no
Isotopes found	High level of IgG	High level of IgM
Memory	yes	Very little or none
Response to carbohydrate antigens	possibly	yes
Response to protein antigens	yes	possibly
Somatic hypermutation	yes	no



A schematic diagram of B cell development:

Hematopoitic stem cell devided and formed progenitor B cell (pro-B cell)

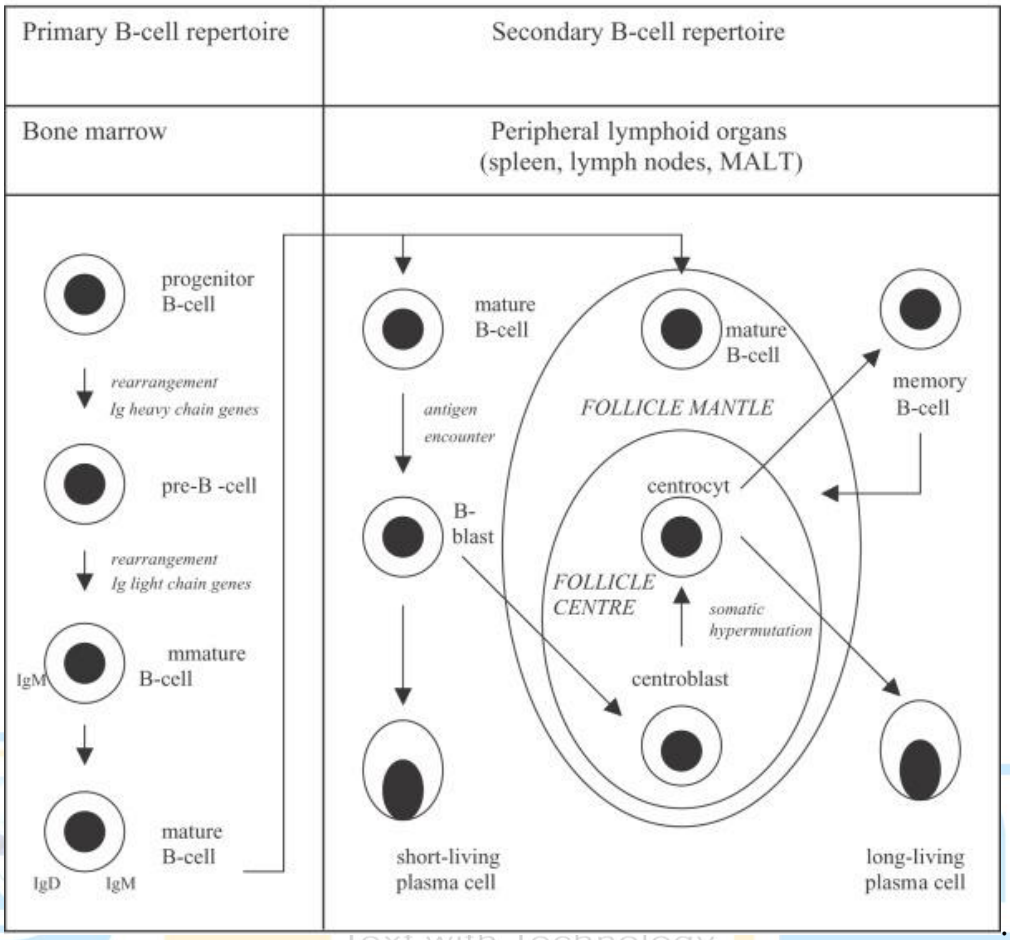
In progenitor stage immuoglobin V region rearrangements occurs. The heavy chains V genes arrange first.

Heavy chain is then expressed on the cell surface in combination with a surrogate light chain and Igα, Igβ signalling complex. Together they form pre B cell.

After light chain rearrangement pre B cell express complete immunoglobin receptor and formed immature B cell.

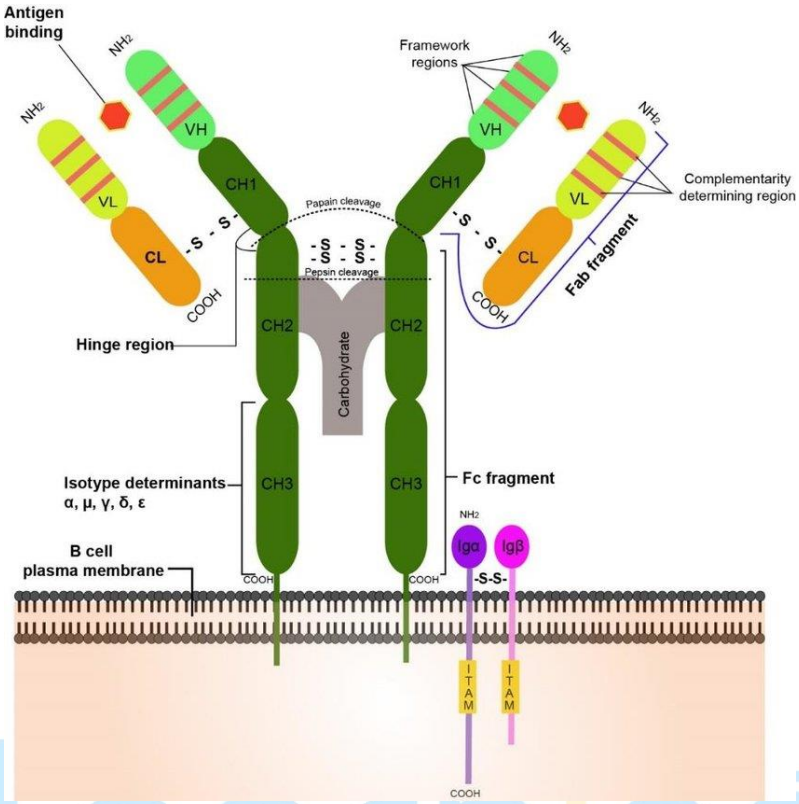
These immature B cell emerge from bone marrow as T1 cell and circulates to the spleen.

T1 B-2 B cell increase the expression of IgD and become T2 cell which matures into either follicular B cell or marginal-zone B cell.



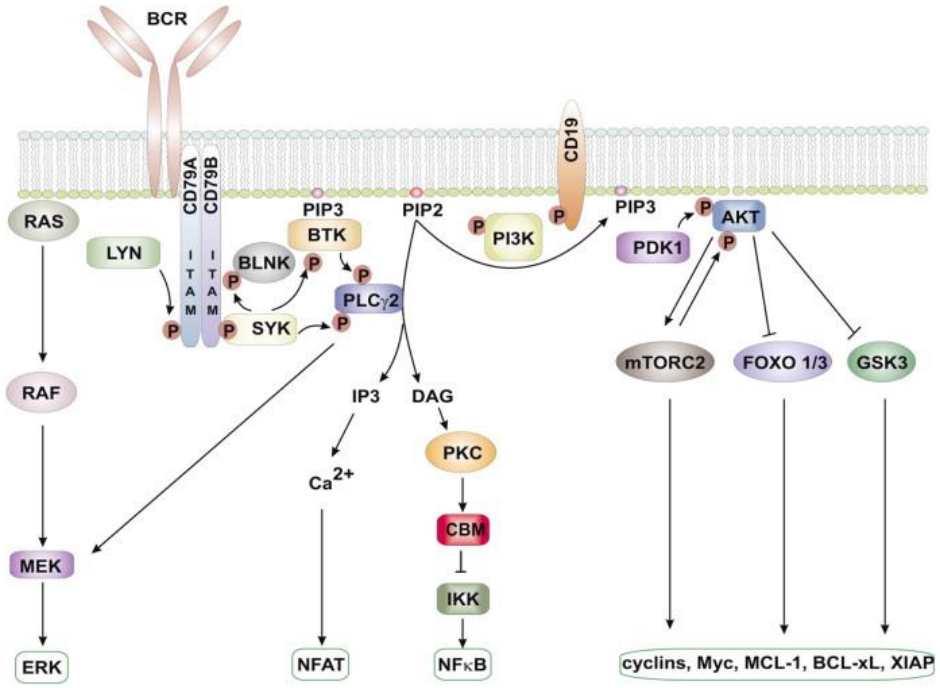
B cell receptors

B cell receptors (BCRs) are the complex which is made up of membrane Ig molecule assotiated with Igα and Igβ. B cell requirs Igα and Igβ to transmit their signals. The coreceptor, CD21 which is associated with CD19 binds to the anigen with a complement molecule C3d. it makes the contanct between antigen and BCR when the antipgen-BCR binding is week. At the cytoplasmic region of the recepor assotiated molecules contain immuno –receptor tyrosine activation motifs (ITAMs). Phosphorylation of tyrosine residued in ITAMs initiates signal transduction. CD19, Igα and Igβ along with CD81 (TAPA-1) helps in signal transduction. T cell receptor also use CD3 (a complex of δε, γε and a pair of ζ or ζν pair). CD4 binds to the MHC classII and CD8 binds to the MHC class I molecule. CD28 binds to the CD80 or CD86 (also known as B7 co stimulatory) to initiate another signal. They are required for stumilation of naïve, but not memory, T c ell.



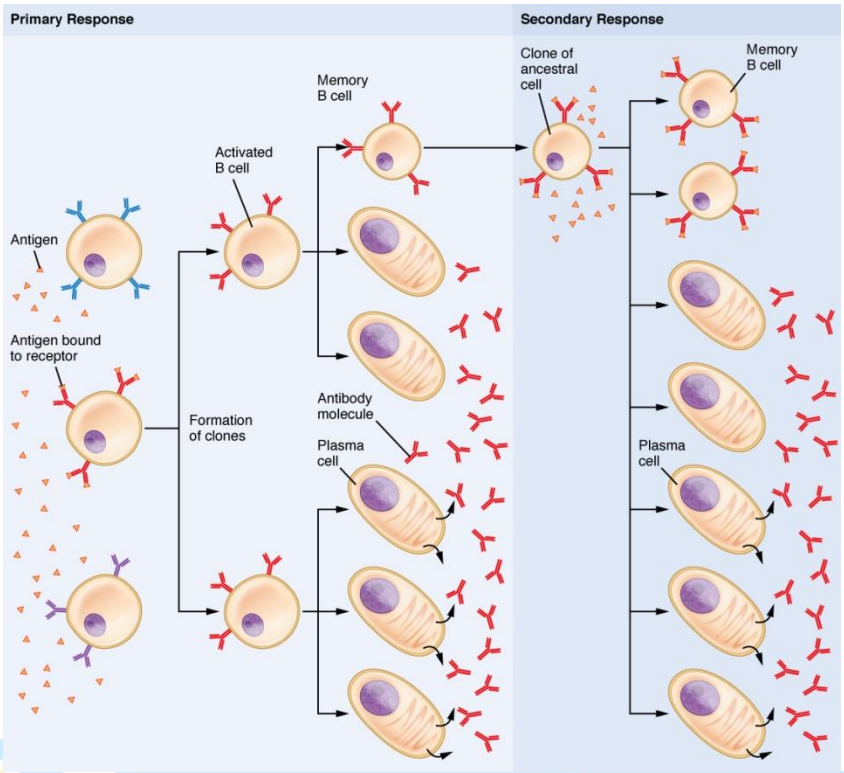
B cell receptor signaling in a schematic diagram:

Binding the BCRs to the antigen induces a comfermational changes which leads to the receptor oligomarization → this antigen mediated receptor clustering leads to the src- kinase phosphorylation of the co receptor Igα and Igβ ITAM residues → this provide an attachment site for the adaptor protein BLNK → an tyrosine kinase, Syk is phophorylated and activated by src kinase → Syk then phosphorylates BLNK and provided docking site for downsream componant and Syk also phosphorylate adaptor protein BCAP and CD19 chich recruita PI3 kinase to the membrane → PI3 kinase helps in fomation of PIP_3 from PIP_2 and recruit ph domain containing protein PDK1 and Akt → phosphorylation by Akt enhance cell survuval and activates the transcrption factor NF-κβ and CREB which supports proliferation differentiation and survival of activated B cells. MAP kinase pathway also activates during B cell activation.



Clonal selection hypothesis

According to the clonal selection hypothesis each B cell bears a single anigen receptor. Antigen interactes with the specific B cell receptor for that antigen. After interaction with antigen B cell proliferates a form a clone of cell which have same identical specificity.every B cell from this clone secrete antibodies which have same specificity as the antigen receptors. At the end of the immune response memory B cell formes which shows a rapid and higher response upon secondary antigen exposure. Those cell bearing the receptors with specificity for self antigens are eliminated from the B cell repertoire during development.



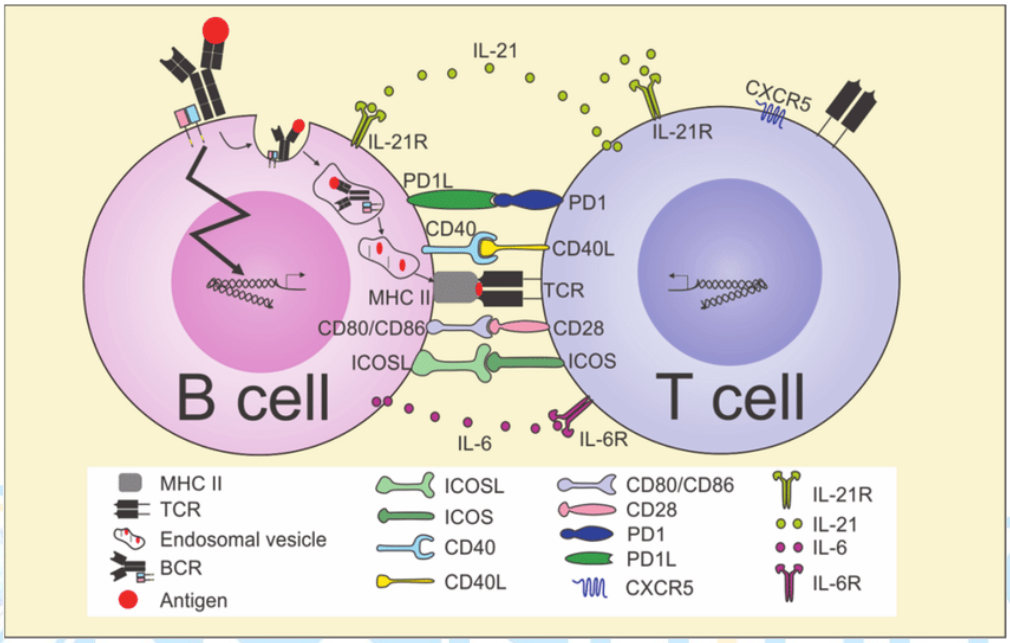
B cell activation

B cell activates by two process : first type of response is generated when the participation of CD4+ helper T cell is require. This is known as T- dependent (TD) response. TD response are mounted by follicular B cell or B-2 B cell. The response of some other antigens does not requires T cell help. These calles the T independent (TI) response. TI responses are mounted by B-1 or marginal zone (MZ) B cell.

T dependent (TD) response:

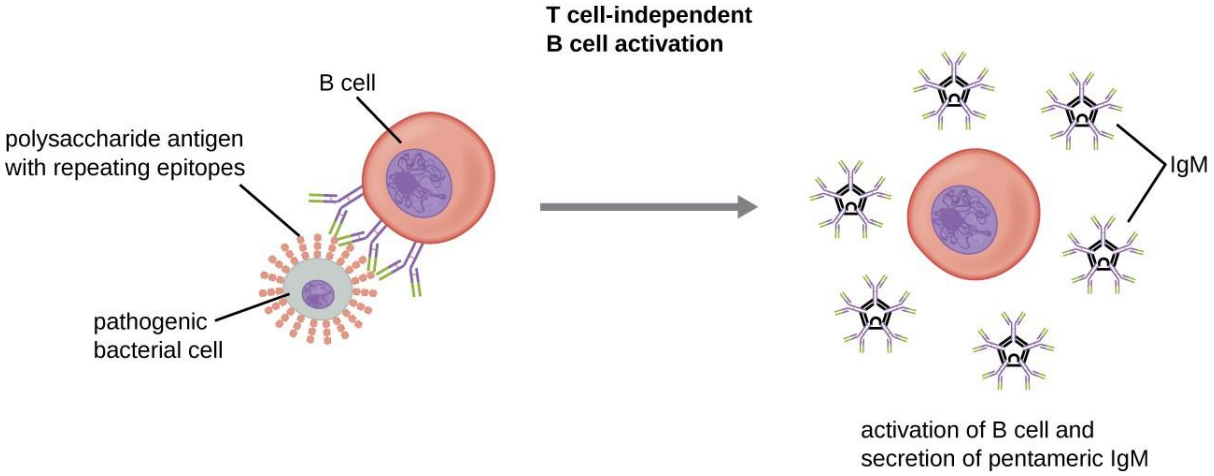
In response to the cytokines CXCL13 B-2 B cell migrates to the lymphoid follicles and survived with the help of survival factor BAFF → either B cell interacts with antigen dirctly or the antigens are taken up first by the subcapular sinus macrophages or follicular dendritic cell and passed on to the B cell → when antigen interacts with BCR the clustering of B cell receptors occur. This is the earlyest phase of activation → this results the internalization of the receptor antigen complex followed by the antigen presentation by B cell to T cell → T cell binds to the B cell by T cell receptor (TCRs). TCR interacts to their cognate B cell by binding to the processed antigen → As well as the interaction between T cell CD28 and B cell CD80 and CD86 , and Tcell CD40L and B cell CD40 occurs →this helps T cell to release cytokines that necessary for full B cell activation →some B cell of antigen stimulated clone quickly differentiate into plasma cell that secrete an initial wave of IgM antibody. This helps in the upregulation of plasma cell transcription factors IRF4 and BLIMP-1. Othe B cell form germinal

centers → in germinal centers high affinity antibodies are formed by generation od somatically hypermutated receptor →both somatic hypermutation and class switching recombination induced cytidinede aminase which helps in DNA repair → at the end of the respone recirculating B memory cell shows highr faster response after secondary exposure and plasma cell reciding in bone marrow continually secrete antibodies that encounter antigen which circulates within the blood.



T independent (TI) response:

TI response is generated by B-1 and marginal zone MZ) B cell. TI anigens interacts with BCR and secreats antibodies relatively low affinity that are IgM class.B-1 B cell derives from a limites numeber of B cell so the antobodies they secreate also less diverse. MZ B cell response to the blood born antigen. B-10 B cell formed interlukins 10 which reduce inflamation.



THE MAJOR HISTOCOMPATIBILITY COMPLEX AND ANTIGEN PRESENTATION

Major histocompatibility factor

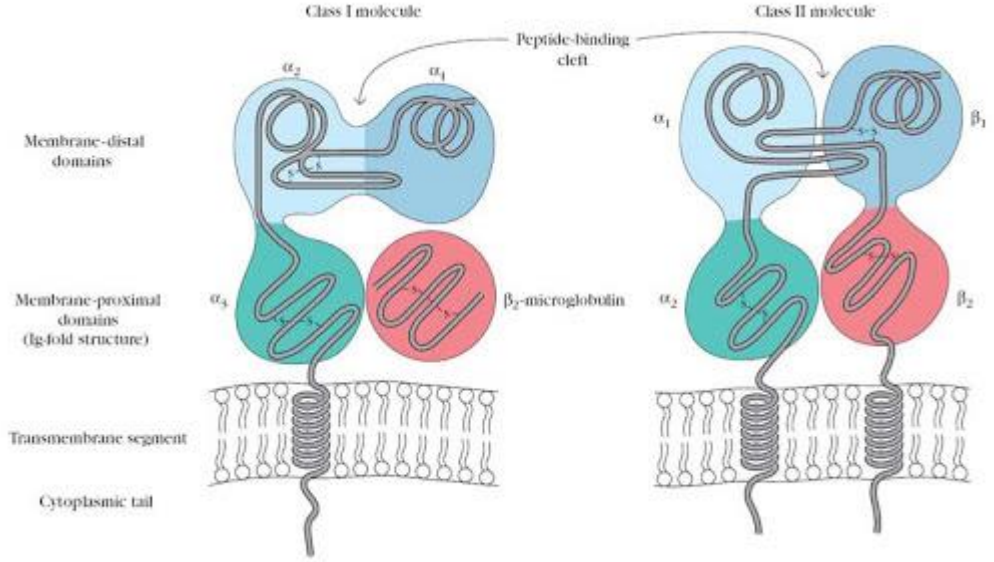
Unlike B cell receptor T cell receptor cannot recognize the antigen alone. TCRs only recognize the antigens when antigen pieces are held within a binding groove of acell surface proteins. These cell surface protins are known as major histo compatibility complex (MHC) molecule. There are three classes of MHC molecule: class I MHC molecule which present in all nucleated cell and present antigen (originate from cytosol,e.g viral proteins) to CD8+ T cell. Class II MHC molecule which expressed almost all subset of leukocytes called antigen presenting cell (APCs) and present antigen from extracellular space (fungi and extracellular bacteria) to CD4+ T cell. Class III MHC molecule which are not participate in immune response. Class I and II MHC molecules shows polymorphism that is a given MHC molecule can bind numerous different peptides. And some peptides can bind to several difference MHC molecule.

Structure of class I MHC molecule:

A Class I MHC molecule formed by assembly of two popypeptides: α chain (45kDa) and β_2 microglobulin (12kDa). The α chain has three domain α1 , α2 and α3. The α1 and α2 interacts and form peptide binding groove. The α chain has a transmembrane domain (25 hydrophobic amino acids) followed by a short charged cytoplasmic anchor segment (30 hydrophilic amino acids). β_2 microglobulin does not contain transmembrane segnment. β_2 microglobulin binds to α3 noncovalently.

Structures of class II MHC molecules:

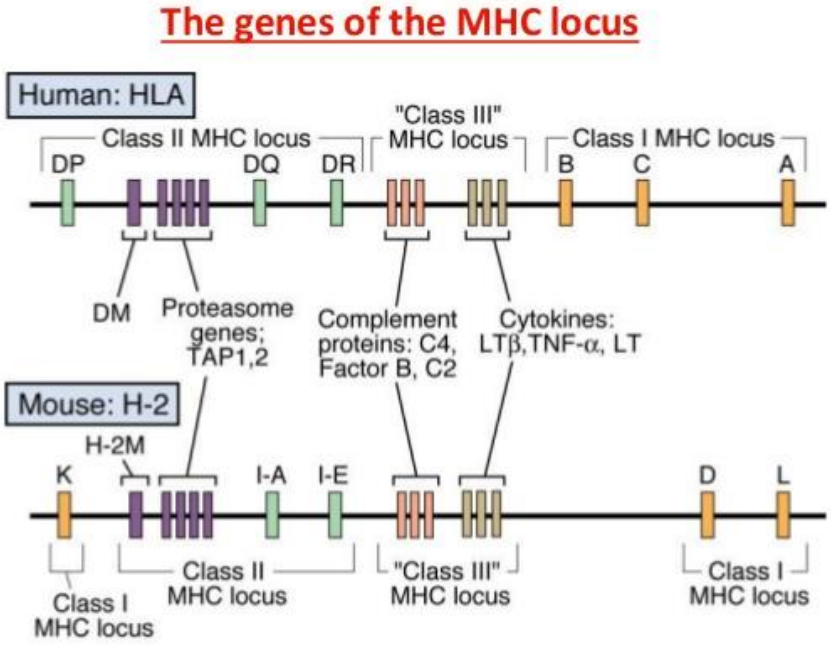
A class II MHC molecule contain two different chains: α chain (33kDa) and β chain (28kDa). They are associated by non covalent interaction. The α chain has two domain α1 and α2 and the β chais also has two domain β1 and β2. The α1 and the β1 formed peptide binding groove.



Inheritance of the MHC

MHC is a collection of gene that present on chromosomes 6 in human and on chromosomes 17 mice. The MHC referredas a human leukocyte antigen complex in humans and as H-2 complex in mice. Class I MHC cell contain two chains α chains and β_2 microglobulin chains. The α chains molecules are encoded by the K ad D region and a additional L region in mice. In human it encoded by ABC loci. β_2 microglobulin is encoded by the gene outside the MHC. Class II MHC molecules are encoded by DP,DQ and DR region in humans and IA and IE region in mice.

MHC molecules are codominantly expressed that means both maternal and paternal gene products are expressed at the same time and in the same cell.



Antigen processing and presentation

In immune system there are two different types of antigens are presents: endogenous antigens and exogenous antigens. Endogenous antigens are processed in the cytosolic or endogenous pathways and presented on membrane with class I MHC molecule. Where as exogenous antigens are processed in exogenous pathway and presen β_2 microglobulinted on the membrane with class II MHC molecule.

Endogenous pathway of antigen processing and presentation: some intracellular proteins are degraded into small peptides by cytosolic proteolytic system call proteasoma which present in all cells. In APCs there is a special type of proteasome presents which is called immuno proteasome. In RER α chain and β_2 microglobulin of class I MHC molecule are formed from

RMA-S cell. Short peptides are transported through a transporter pretein TAP(for transporter associated with antigen processing) in rough endoplasmic reticulum (RER). Then the assembly of these components with class I MHC molecules occurs which inclides the participation of molecular chaperons that facilites the folding of polypeptides. The α chaind of class I MHC molecules binds calnexin and ERp57. When β_2 microglobulin bind to α chain calnexin dissociates ans the claaI MHC molecule associate with calreticulin and tapasin. Tapasin brings the TAP transporter in a proximity with class I MHC molecule which captures the peptide by the the help of TAP protein and exposed in luminal environment. ER aminopeptidase (ERAP 1) removes the amino terminal resideus to accure the length of class one binding size. Calreticulun, tapasin and ERp57 dissotiates and the class I MHC Molecule with peptide ex press on cell surface via glogo complx.

Exogenous pathway for antigen processing and presentation:

Antigen presenting cells (APCs) internalized the exogenous antigen by sevaral process like phagocytosis, receptor mediated endocytosis, pinocytosis. After intenalization of antigens they are appares to involve several increasing acidic envirenments i.e, early endosoma (pH 6.0-6.5), late endosome (pH 4.5-5.0) and lysosome (pH 4.5).In late endosome of APCs final degradation of peptides and the peptide binding to the MHC II molecule occures. Class II MHC molecules are formed in RER with class I MHC Molecules. The α and β chain of class II molecules associted with a protein calld invarient chain which present in the peptide binding groove and preventing the binding of endogenous antigens. This class II MHC molecule with invarient chain transport from RER to late endosome through Golgi complex and early endosome. By proteolytic activty of this compertment invarient chains degreaded leaving a small fragment called CLIP which remain binds with the peptide binding groove. HLA-DM a nonclassical class II MHC molecule helps in the exchange of CLIP for antigenic peptide. Once the peptide bins to the class II MHC molecule they transported to the plasma membrane where in neutral pH they become more compact and stable.

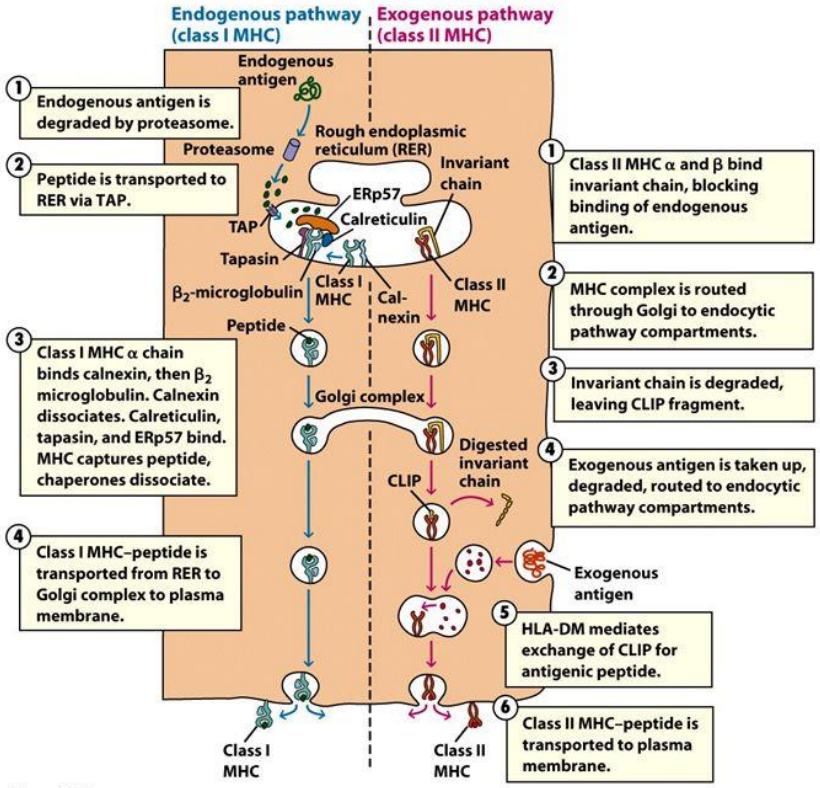


Figure 8-23
Kuby IMMUNOLOGY, Sixth Edition

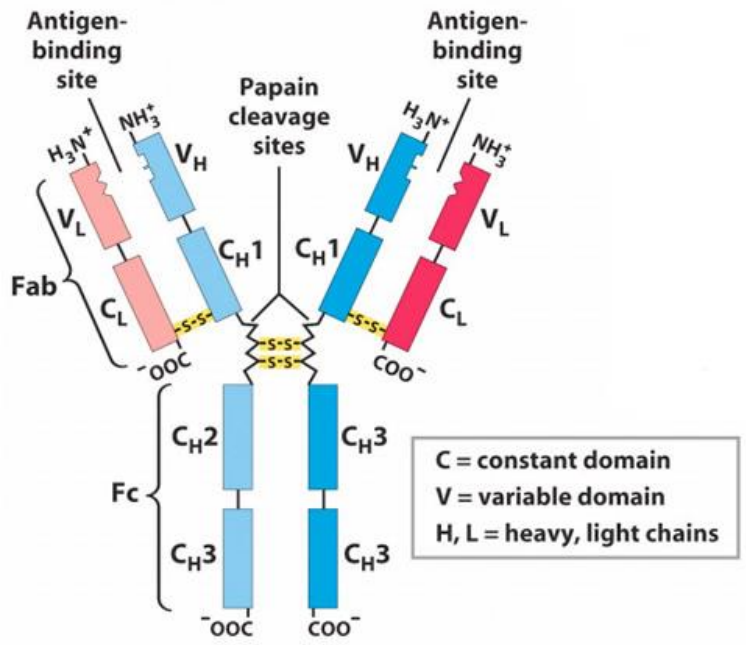
Immunoglobulins

Antibodies secreted by B cellsand treir membrane bound receptor forms belobg to the immunoglobulin family of proteins. They have one or mone immuno globulin domeins. In antibodi molecules immunoglobulin domain made up of approximately 110 amino acids and each β sheet contains three to five strands.

Structure of antibodies:

All antibodies have two chains : two identical light (L) chains and two identical heavy (H) chains. Light chains have two region one is variable or V_L region and another is less variable or constant region C_L region. This constant region of light chain formed by κ or λ chains. In humans 60% of light chains are κ and 40% of light chains are λ. This λ chains are further devided into four subtypes— λ1,λ2,λ3 and λ4. In varialble region of light chain there is a region which is hypervariable called complimentary determinig region (CDRs). The heavy chains also have a variable region V_H and a constant region C_H1. The heavy chains constant region have five basic patterns: μ, δ, γ, ε and α . they are reffered as isotopes and they devided antibodies in five different class. IgM antibody with a heavy chain of μ isotype IgD with δ isotype, IgG with γ isotype, IgE with ε isotype and IgA with α isotype. Each light chains and heavy chains are bound by disulfide bond between cysteine residues of V_H and V_L domain and C_H1 and C_L domain. Multiple disulfide bonds are found in the heavy chain and c terminal portion of heavy

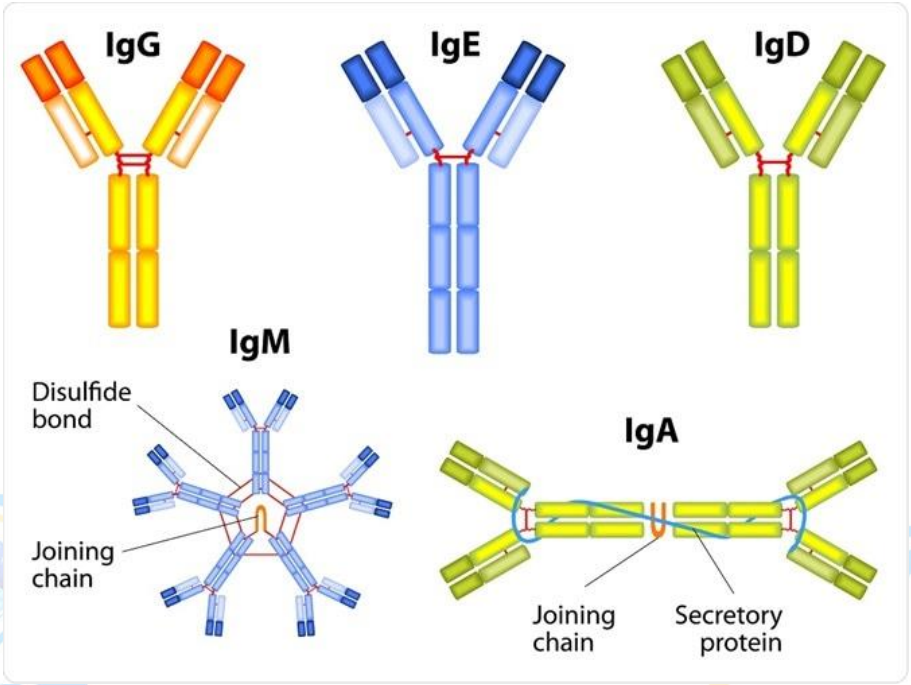
chain also participate in noncovalent bonding. Antibosy molecules are 'Y' shaped and antigen binding regions are at the tip of 'Y'. Antibody has a more flexible hinge region where three regions (two Fab and one Fc region) are joined. A proteolytic cleavage by the enzyme papine devided the antibody molecule into two identical fragments that have antibody binding specificity called Fab region and the remaining region which is identical for all antibodies and cryatallizes easily known as Fc region. Fab region bins to the antibodies and the F c region bind to the Fc receptor on phagocytic cell.



classification and chain composition of five immunoglobulin classes:

Class	Heavy cahins	Light chains	J chains	Number of C_H Ig domains	subclasses	Molecular formula
IgM	μ	κ or λ	yes	4	none	$(\mu_2 \kappa_2)_n$ $(\mu_2 \lambda_2)_n$ n= 1 or 5
IgG	γ	κ or λ	none	3	γ1,γ2,γ3,γ4 (human) 1,γ2a,γ2b,γ3 (mouse)	$\gamma_2 \kappa_2$ $\gamma_2 \lambda_2$
IgA	α	κ or λ	yes	3	α1,α2	$(\alpha_2 \kappa_2)_n$ $(\alpha_2 \lambda_2)_n$ n= 1,2,3 or 4

IgD	δ	κ or λ	none	3	none	$\delta_2 \kappa_2$ $\delta_2 \lambda_2$
IgE	ε	κ or λ	None	4	none	$\varepsilon_2 \kappa_2$ $\varepsilon_2 \lambda_2$



T CELL — DEVELOPMENT, ACTIVATION, DIFFERENTIATION AND MEMORY

T cell develops in two cluster of events : one is early thymocytes developments and the second is selection precedure.

Early thymic develpoment: uncommited WBCs progenitor enters in to the thymus through blood vessel from bone marrow. There is a receptor, Notch, which have been clasically associated with enbryonic scell development are reqired for T cell commitment. Development of T cell is a very organized process and it takes place in distinct microenvironment. T cell first enter into the thymic cortex and then in thymic medula and exits through cortico medullary junction. Earliest T cell does not have detectable CD4 and CD8 so they are called double negetive (DN) cell. DN t cell have four sub types DN1,DN2, DN3, DN4 depending on the presence or absenc eof other cell surface molecule. DN 1 thymocytes first enter into the thymus and they proliferate and become DN 2 thymocytes in subcapsular cortex. DN2 then transfer into the DN3 thymocytes which successfully rearrange the TCR β chains via the process β selection. DN3 enters in ti the final stage i.e. DN4 which matures directly into CD4+ CD8+ DP thymocytes. B selection process initiate an assembly of TRCβprotein with a surrogate, TCRα chain and CD3. Pre TCR (TCR/CD3) initiates a signalling pathway. DN4 T cells mature

and start rapid proliferation. CD4+ and CD8+ double positive (DP) cell developes, and the proliferation ceased which leas to the initiation of TCRα chain rearrangements.

DN thymocyte type	Location	description
DN1	Bone marrow to thymus	Migration to thymus
DN2	Subcapsular cortex	TCRγ,δ and β cell rearrangement and T cell linage commitement
DN3	Subcapsular cortex	Epression of pre –TCR and β selection
DN4	Subcapsular cortex to cortex	Proliferation \, allilic exclution of β chain locus, α cahin locas rearrangements begins, become DP thymocytes.

Thymic Selection procedure

DP thymocytes are most abundant in thymas which bears TCRαβ receptor. These DP cells are goes through the t hymic selection procedure. This thymic selectionare held by two process: positive selection and negetive selection.in positive selection those thymocytes are selectes which bears the receptors capable of binding self MHC molecules resulting the MHC restriction. Negetive selection, selects against thymocytes bearing high affinity receptors for self –MHC/ peptide complex resulting self tolarence. The large number of DP thymocytes (95%) do not interact with any MHC/ self peptide expressed by thymic epithellium and die by neglet. After that linage commitment procedure starts where DP cell can decide weather to become a helper T cell or cytotoxic T cell. DP T cell which can interact with class II MHC molecule continues the signals that initiate the formation of CD4+ helper T cell. DP cell that can interact with MHC I molecule initiate the signalling for formation of CD8+ cytotoxic T cell. DP cell transfer into the medulla and transfer into the SP cell. This process is negetavily selected for tissue specific antigen in medulla. The fully mature thymocytes are leaves thymus and undergo final maturation in peripheral lymphoid organ.

TCRαβ lineage comprises the majority of T cells

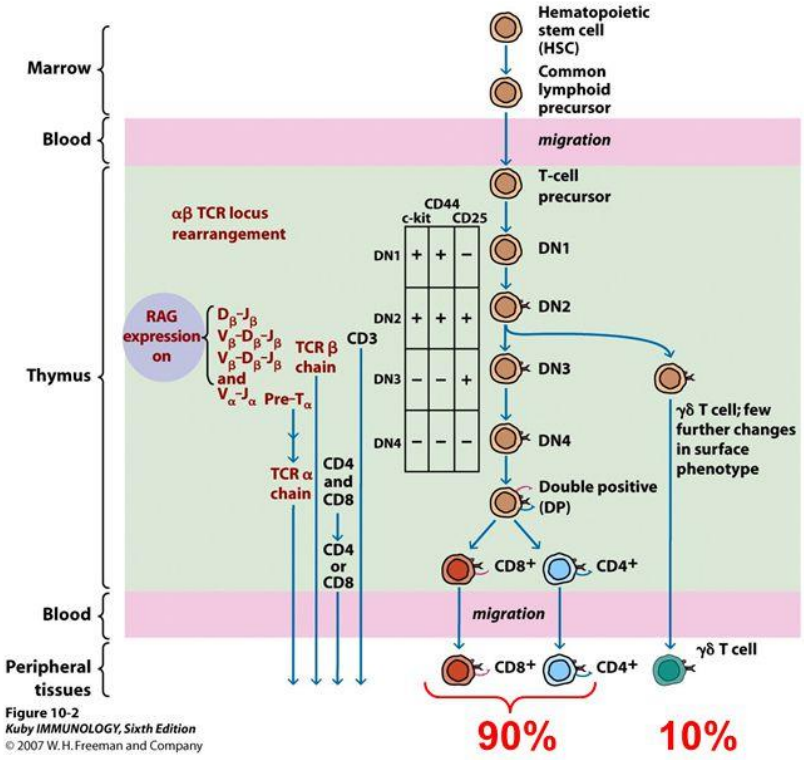
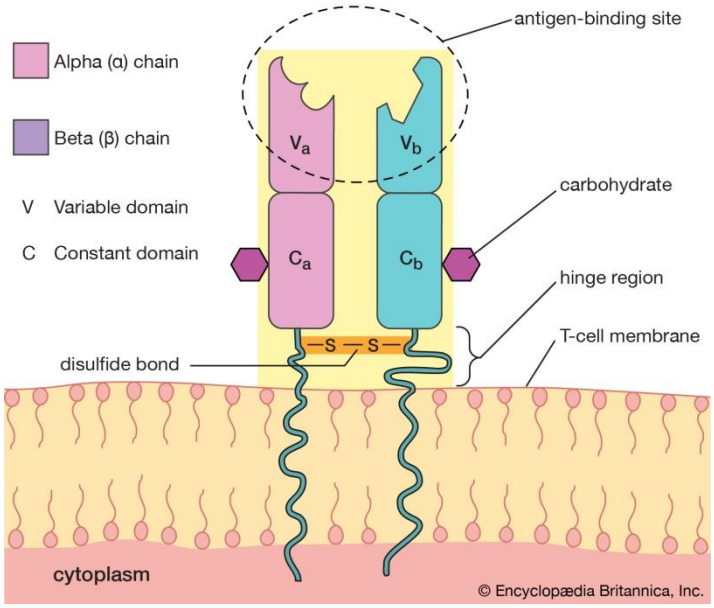


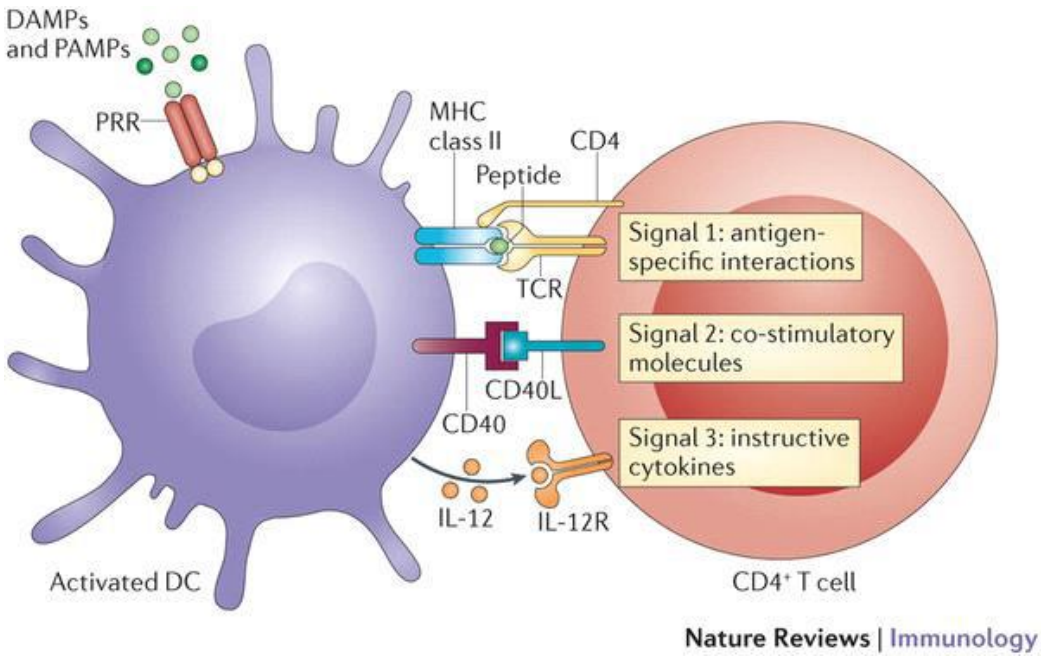
Figure 10-2
Kuby IMMUNOLOGY, Sixth Edition

T cell receptor: T cell receptors are heterodimer and consist of α and β chai which recognizr the complex antigens. Some T cell receptor made up of γδ chains. Like Igα/Igβ in BCR, TCR also have a complex protein called CD3. CD3 made up of three dimers: δε pair,γε pair and either two ζ or ζη pair. Cytoplasmic tail of CD3 also have ITAM sequence which helps in TCR mediated signalling pathway. TCR non covalently associated with many cell surface molecule which is known as co receptor. CD4+ and CD8+ are the co receptors. CD4+ interacts with the MHC class II molecule whereas CD8+ interacts with MHC class I molecule.



T cell activation

T cell activation is a part of adaptive immune response. It initiates when a naïve T cell interacts with APCs specially dendritic cell. Three signals are required for T cell activation: signal 1, initiates when TCR-CD3 interacts with MHC peptide complex on a dendritic cell. Siganl 2 initiates by the interaction of B7 molecule expressed by APCs and co stimilatory CD28 or ICOS expressed by T cell. Absence of this signals leads to the clonal anargy or t cell inactivity. Signal 3 initiates by the releasing of cytokines that plays a effective role in determining of effector cell that a T cell become.

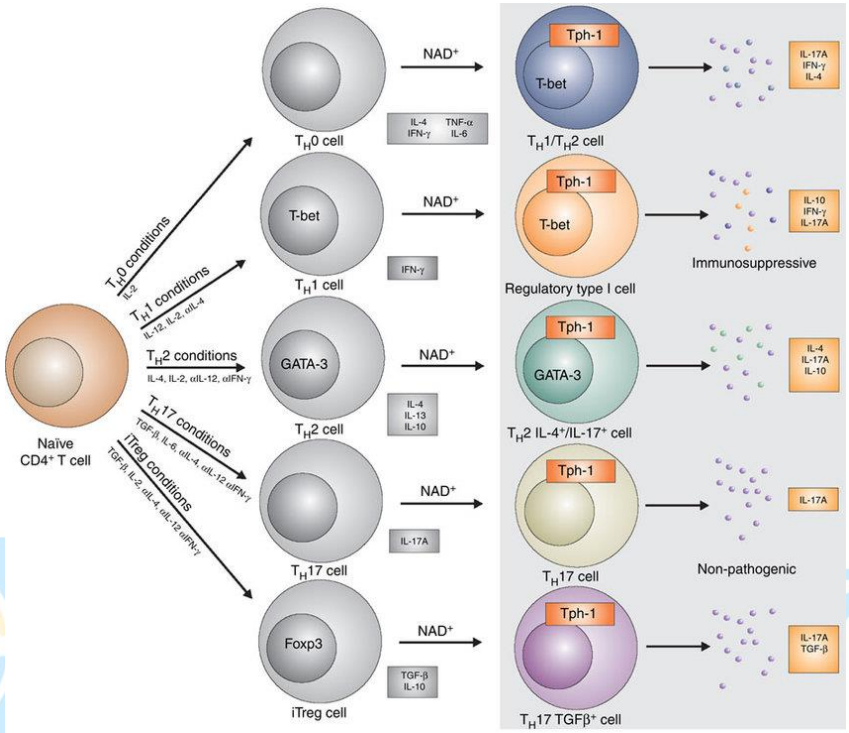


T cell diffrentiation

Activated T cell become differentiate into effector helper or cytotoxic T cell. Helper T cell express CD4+ and recognize MHC class II Molecule where as cytotoxic T cell express CD8+ and recognize MHC class I molecule . CD4+ helper t cell further devided into five mail subpopulation: T_H1, T_H2, T_H17, iT_{REG} and T_{FH}. Each subpopulation have unique set of polarizing cytokines that initiates differentiation, transcriptional regulators helps in production of helper T cell gene and effector cytokines that they secrets to regulates immune response.

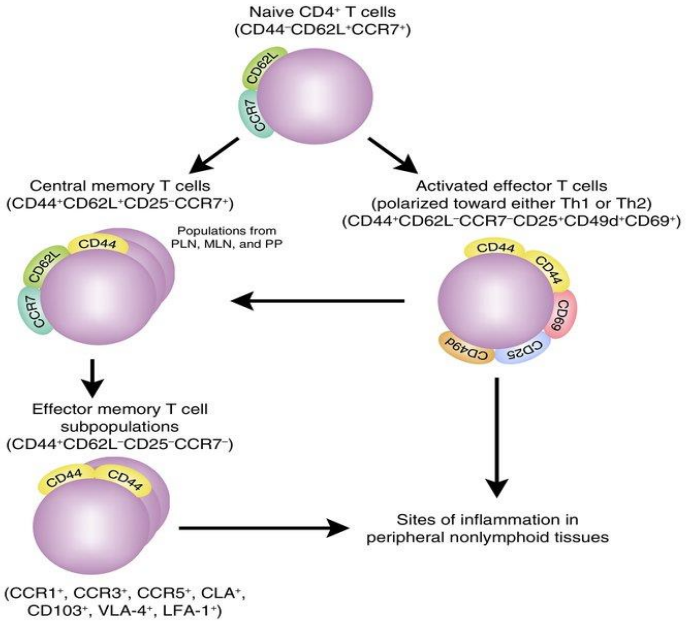
Cell type	Polarizing cytokines	Master gene regulators	Effectors cytokines	Function
T_H1	IL-12, IFN-γ, IL-18	T-Bet	IFN-γ, TNF	Enhance APC activity Enhance T_c activation Protects against intracellular pathogens Involve in the delayed type of hypersensitivity and autoimmunity
T_H2	IL-4	GATA-3	IL-4, IL-5, IL-13	Protects against extracellular pathogens Involve in allergy
T_H17	TGF-β, IL-6, IL-23	RORγ	IL-17A, IL-17F, IL-22	Protects against some fungai and bacterial infection Contributes to imflamation and autoimmunity

iT_{REG}	TGF-β, IL-2	FoxP3	IL-10, TGF-β	Inhibites inflamation
T_{FH}	IL-6, IL-21	Bcl-6	IL-4, IL-21	B cell help in follicle and germinal centre,



T cell memory:

There are two types of memory T cells are found : one is central memory (T_{CM}) cell and another is effector memory (T_{EM}) cell. Central memory cella are long lives and they are stored in the secondary lymphoid organ and they can produced different effector T cells. Effector memory cells present at the site of infection and immidiately reexpress the immune response against the antigen.



CELL COMMUNICATION

CELL JUNCTIONS

We can also call the cell junctions as the intercellular bridges, they the special type of cellular components which are able to contact or communicate with the neighbouring cell or with the extracellular matrix. Their abundance mainly noticed in the epithelial tissues. They also play an important role in stress reduction, placed upon the cells.

There are mainly three types of junctions, known as: Occluding junctions, Anchoring Junctions and Communicating Junctions.

OCCLUDING JUNCTIONS

These are the junctions seals the cell together in a single epithelium, following a special way, which prevent the leaking of molecules from one side to another of a cell. This junction is basically divided into two types, phylogenetically. They are as follows:

TIGHT JUNCTION

These cell -cell occluding junctions present mainly in vertebrates and are basically multiprotein complexes which seals the paracellular pathway by preventing transport of molecules.

Their structure is associated with two main transmembrane proteins **Claudin** and **Occludin**, which are associated with the peripheral membrane protein **ZO** (zona occludens).

Occludins are discovered 1st, are the 60kDa proteins with four transmembrane domains with both N and C termini. Caudins are discovered later which is of 20kDa and of approximately similar structure. There is some **JAM** (Junction Adhesion Molecules) which are the part of immunoglobulin superfamily, and their weight is about 40kDa.

The main function of these junctions is to prevent the free passage between the cells and to maintain the cell polarity by preventing the diffusion of molecules across the apical and basolateral surface.

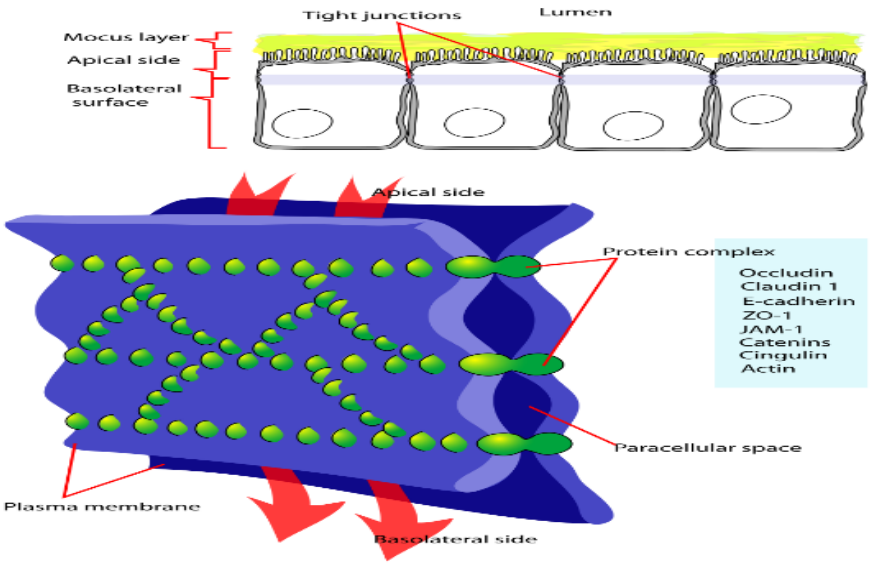


Fig.: Image of a tight junction

SEPTATE JUNCTION

These are found mainly in invertebrates. They show a ladder like structure with the same functionalities like tight junctions in vertebrates. There are a lot of components maintains the structural and functional activities of septate junctions, like: Band4.0 coracle, Disc large, Neurexin IV, Fasciclin iii etc. There are three known claudin proteins in it: Mega, Sinu and Kune. These junctions are also found in the myelinated nerve fibre of vertebrate.

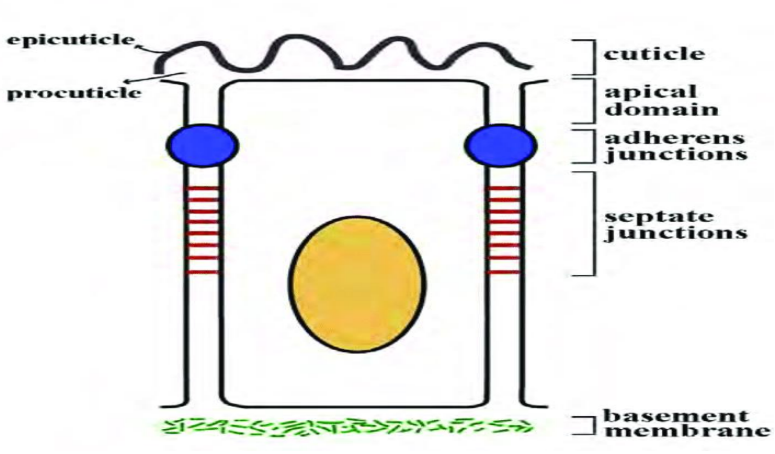


Fig.: Image of a septate junction

ANCHORING JUNCTIONS

They are second category of cell junction molecules. These types of junctions attach the cells to their neighbouring or to the extracellular matrix and also perform an important role to join the cell together into tissues.

They are basically of three types: Adherens Junction, Desmosome Junction, Hemidesmosome Junction.

ADHERENS JUNCTION

They basically connect the bundles of actin filaments from cell to cell or cell to ECM. They show two types of connections mainly:

1) Adhesion Belt or Zonula Adherens: Actin filaments + Cadherin family protein
   (located near the apical surface, bellow the tight junction)
   Other helper proteins are – P120 (delta catenin), Plakoglobin (gamma catenin), alpha catenin, beta catenin, vinculins.

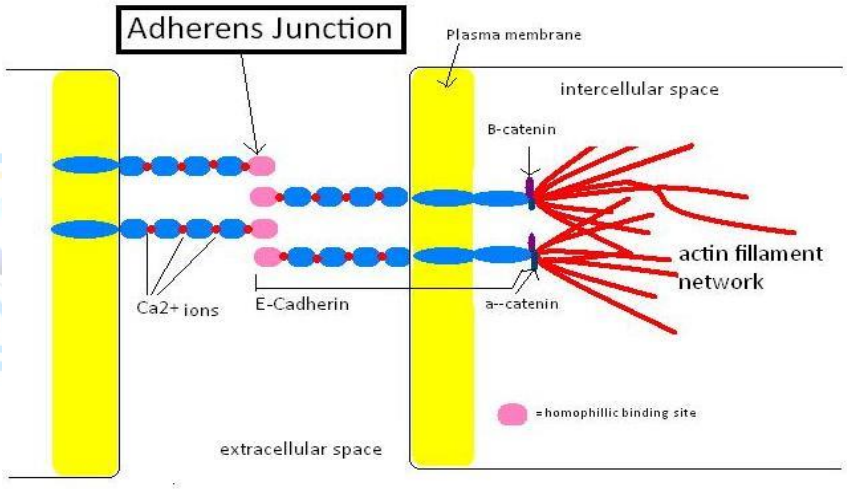


Fig.: Image of adherens junction

2) Focal Contact or Adhesion Plaques: Actin filaments + integrin family transmembrane proteins.
   (located in the cell ECM junction)

DESMOSOMES

They are also known as macula adherens and **nodes of bizzezero** (Italian pathologist Guilio Bizzezero discovered it first). They are one of the stronger cell adhesion molecules randomly arranged at the lateral side of plasma membrane.

It is basically dense cytoplasmic plaque present in both connecting cells- composed of proteins (like, Plakoglobins and Desmoplakins). They are further connected with two structures- one is intermediate filament and another is linker transmembrane proteins of cadherin family.

Intermediate filaments are of two types: keratin filaments (for skeletal muscle) and Desmin filaments (for heart muscle). And the linker TM protein of cadherin family is connected with the TM domain of another cell.

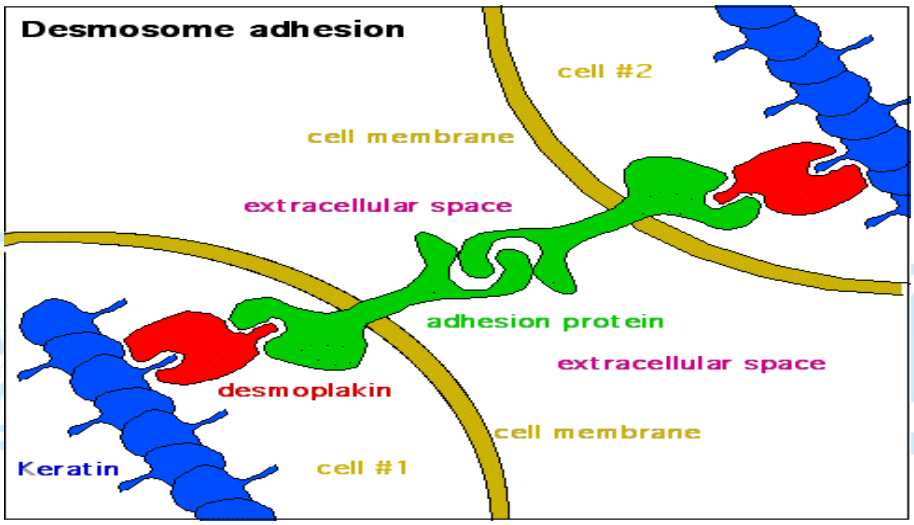


Fig.: Image of desmosome

HEMIDESMOSOME

These are basically called the half desmosomes (integrin TM family). They not only join the adjacent epithelial cell membranes of two cells but also connect the basal surface of epithelial cell to basal lamina (a floor where the epithelium and connective tissue connects). It has two membrane spanning components- integrin α6β4 and plectin 1a. They are subdivided into two types – type I hemidesmosome and type II hemidesmosome.

Their involvement is found in signalling pathways like keratinocyte migration, carcinoma intrusion.

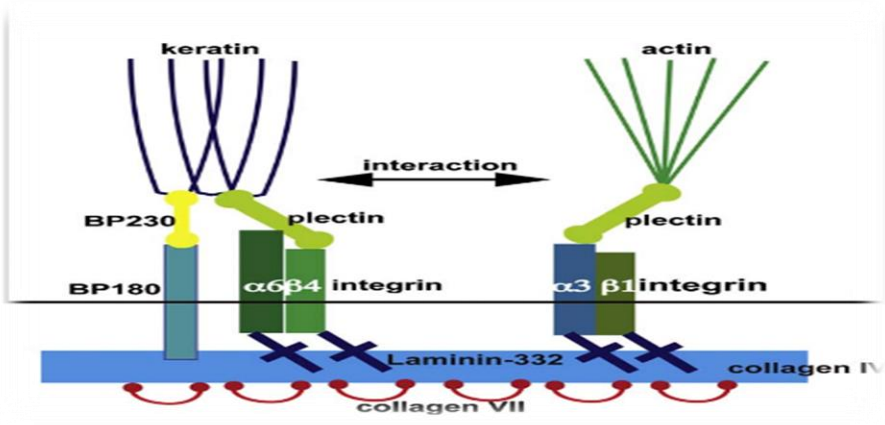


Fig.: Image of hemi desmosome

COMMUNICATING JUNCTION

They are another special type of junctions which helps in the movement of chemical and electrical signals from one cell to its partner cell. This is basically of two types: Gap junctions and Plasmodesmata.

GAP JUNCTON

These junctions form direct connection with adjacent cell. They basically act as a channel and allow the particles to be entered into cell with respect to some considerations like particles must be within 1000Da and water soluble in nature.

Gap junctions allow chemical signals in form of ions to excite cells. (example: heart muscle cell). Gap junctions electrical signal transmission in case of nerve cells.

Gap junctions consists of special transmembrane proteins, called **Connexins**. Six connexions are joined together to form a barrel like structure, called **Connexon**. When the connexion of different cells come in contact, a loop is formed in between the structures, which allows the particles to transfer.

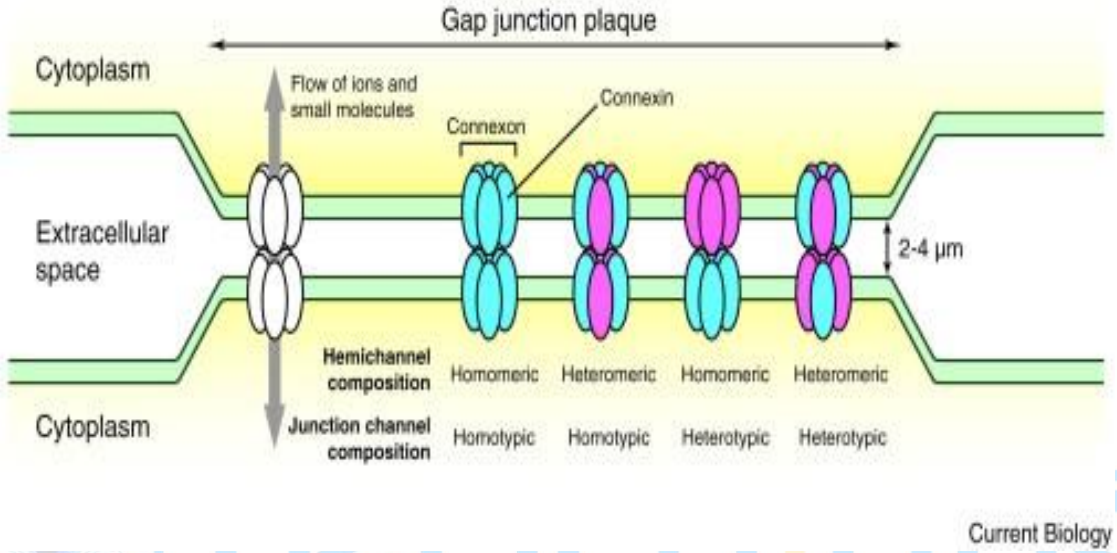


Fig.: Image of gap junction

PLASMODESMATA

They are called Plasmodesma in singular and mainly seen in case of plant cells. At each plasmodesma position the cellular plasma membrane is continuous with other plasma membrane creating a open channel in between the two cytoplasm. A smooth endoplasmic reticular extension, called Desmotubule passes through thee cytoplasmic pore, and creates a dual sided ring. The middle part of two rings are formed with the help of some proteins called PCP or Plasmodesmata Channel Protein, which allows small molecules to pass.

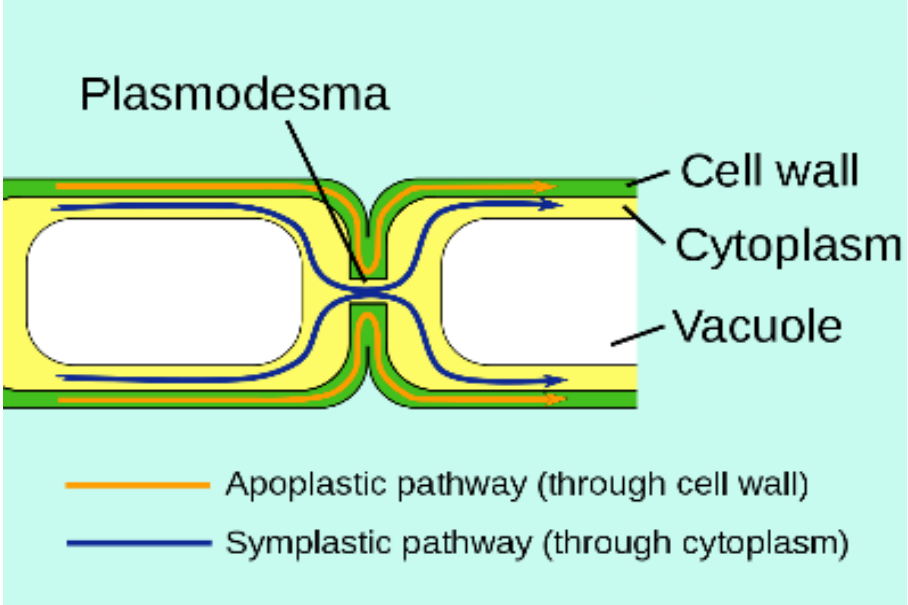


Fig.: Image of plasmodesmata

CELL ADHESION MOLECULES (CAM)

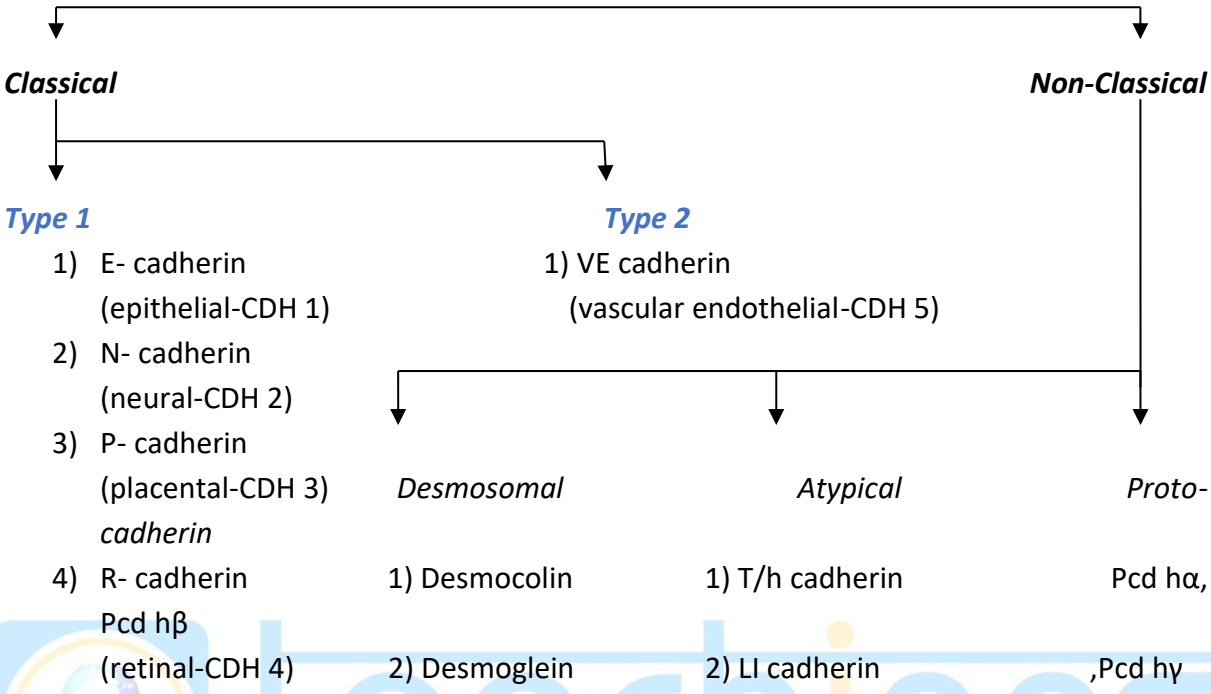
They are the molecules helps in the connection between cells or the attachment of cells substrate. They are basically transmembrane proteins or glycoproteins in nature. According to the subject of attachment they can be divided into two types: cell to cell adhesion and cell to matrix adhesion

CELL-CELL ADHESION: There are several groups of proteins helps in the cell-cell adhesion procedure like- cadherin, selectin, immunological super family etc.

CADHERINS

Cadherins are one of the important types of cell adhesion molecules, which promotes to form adherens junction. These are basically calcium dependent molecules and composed of 700-750 amino acids. They are basically associated with homophilic cell-cell interaction through their ECF part and get connected with the cytoskeleton via a number of anchor proteins like- α catenin, β catenin, vinculin, plakoglobin etc through their cytosolic part.

CADHERIN



SELECTIN

They are also called CD 62. They are transmembrane proteins but with a specialization of conserved lection domain. They mediate cell-cell adhesion by heterophilic binding and just like cadherin it also depends on the ECF calcium ion concentration to function. They are basically of three types, as follows:

L- Selectin: It is the smallest among the vascular selectins, expressed in all granulocytes-monocytes- lymphocytes and mainly in the leucocytes. It functions as a homing receptor. Commonly found in T cell surface also.

P- Selectin: It functions on the surfaces of activated endothelial cells and platelets. It is stored in granules, inside the deactivated platelets known as Weibel Palade Granules. It has different names like CD62P, GMP 120, PADGEM etc.

E- Selectin: This type of cell adhesion molecule only expressed in endothelial cells. It shows an important role in inflammation and cancer. It is also known as CD62E, ELAM 1 etc.

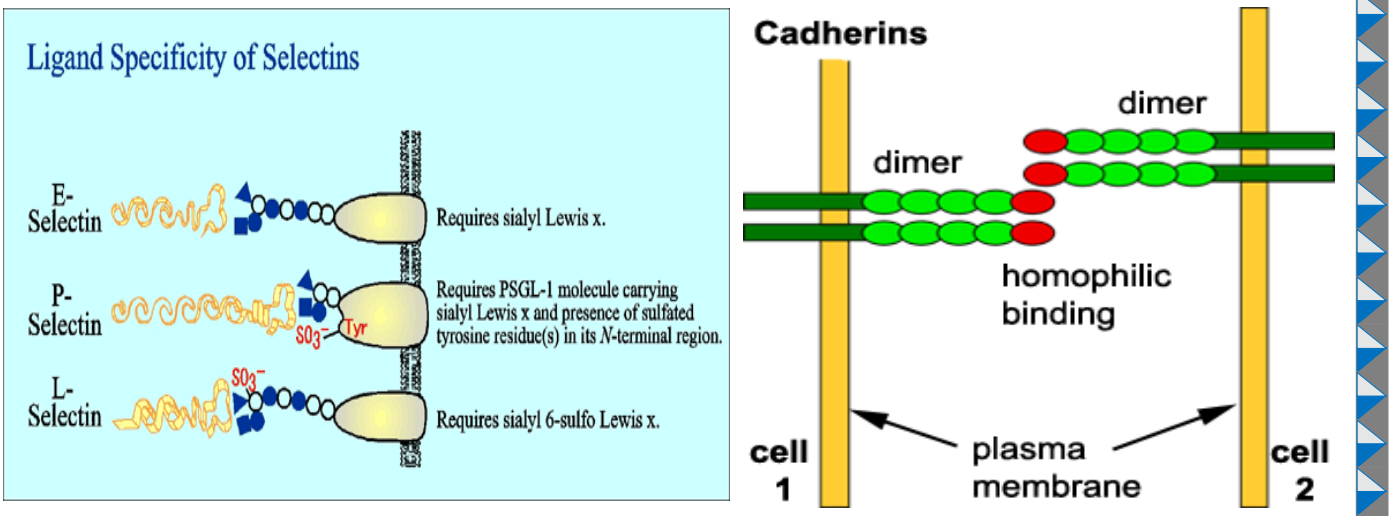


Fig.: Image of selectin

IMMUNOGLOBULIN SUPERFAMILY

They are important types of cell-cell adhesion molecules, which are not calcium dependent. The members of this family are defined by, presence of one or more immunoglobulins in their surface. They can exhibit both homophilic and heterophilic connection.

They are basically of three types: ICAM (intracellular cell adhesion molecule), VCAM (vascular cell adhesion molecules) and NCAM (neural cell adhesion molecules). Among them ICAM and VCAM are responsible of heterophilic type of binding, which act on endothelial cell and intigrins of WBC. The 3rd type member is responsible for the homophilic type of binding which acts on various cell types.

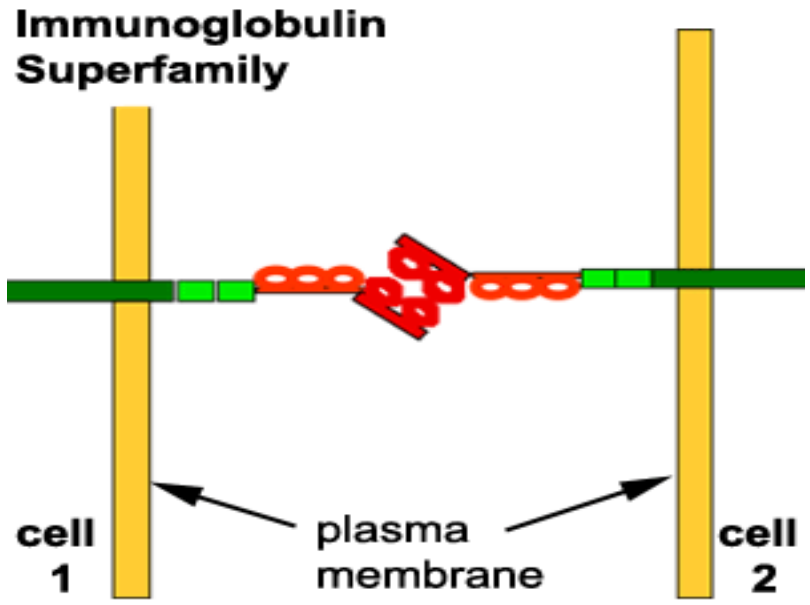


Fig.: Image of a common Ig family adhesion molecule

CELL MATRIX ADHESION

INTEGRINS

These belongs to the transmembrane glycoprotein family and the principle cell matrix adhesion molecule. Their structure is composed of two non-covalently associated transmembrane glycoprotein subunits, alpha and beta. The extracellular alpha beta region is the cation binding domain part in which ligands come to bind. The intracellular carboxyl domain part is attached with several kinds of adaptor proteins (like Talin, alpha actinin, venculin etc), which are further associated with a network of actin filaments.

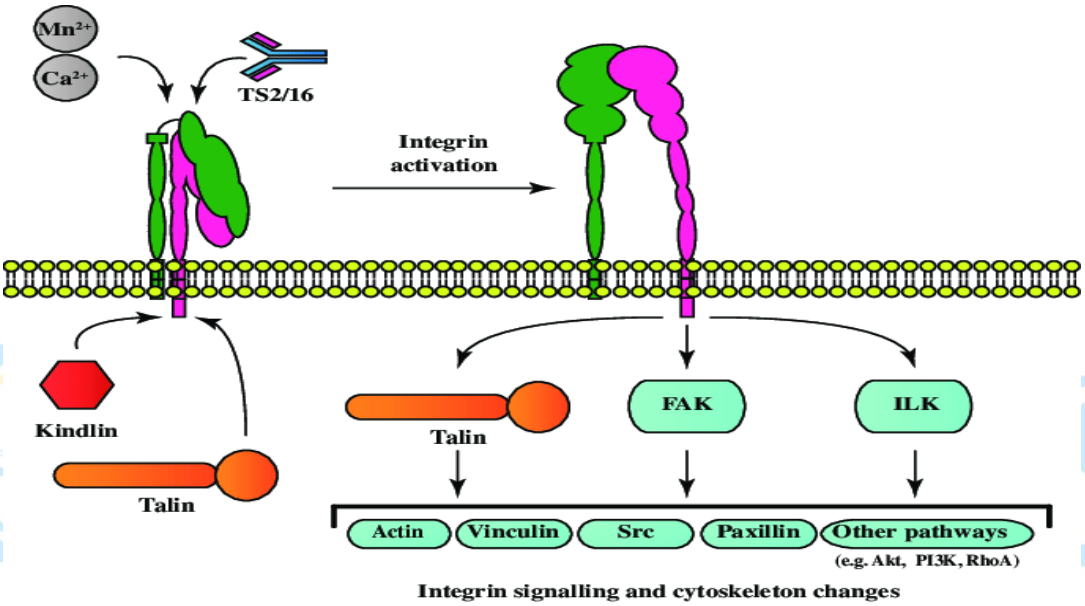


Fig.: Image of a common integrin signalling

EXTRACELLULAR MATRIX

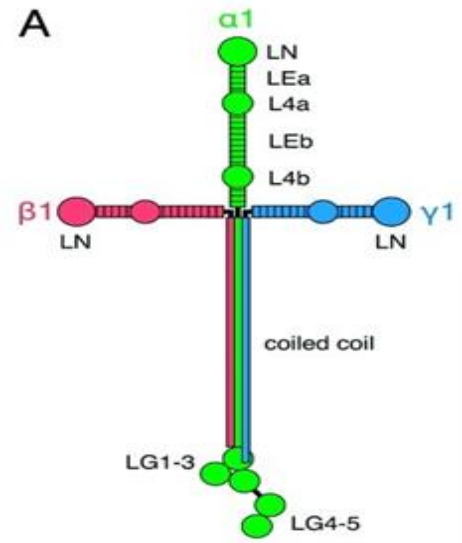
The extra cellular matrix region of cell can be divided into several groups, like-

1) **Structural protein**: Collagen is one of the major structural protein of this section. It is the single most abundant protein in the tissue level also, though they may contain another protein (connective tissue contains Elastin protein).
2) **Proteoglycan**: Glucosaminoglycans (GAGs) are mainly found in extracellular matrix, covalently linked to proteins in form of proteoglycans. Proteoglycans differ from the glycoproteins. GAGs are basically repeating units of disachharaides (N-acetyl glucosamine or N-acetyl galactosamine). Among the disachharaides one must be sulphated another must be an acid (like, glucoronic acid or iduronic acid). There are various types of GAGs, like- condrin + dermatan sulphate, heparin sulphate, keratin sulphate, hyaluronan sulphate (simplest).

3) **Adhesion Proteins**: There are mainly two types of adhesion proteins are found in the extracellular matrix, and they are laminins or laminin proteins and fibronectins.

LAMININ

These are the heterotrimeric glycoproteins, mainly composed of three polypeptide chains α, β, and γ, which are interconnected through disulphide bonds. All the α, β and γ chains have separate N and C terminal ends, among them the α chain is the central part and the β, γ chains are surrounded over the α chains in a spiral fashion. The α chain contains LG domains – LG 1,2,3 domains are responsible for integrin binding and LG 4,5 domains are responsible for proteoglycan binding domain (α-dystroglycan). All the LG domains have calcium binding specificity.



The main function of laminin proteins are organisation and establishment of basal lamina, their primary function is to cell matrix attachment and other important functions are cell growth, migration, tumour growth, nerve regeneration etc.

FIBRONECTIN

These are one of the important extracellular matrix glycoproteins, found in all vertebrates. These proteins help in the attachment of cell to extra cellular matrix by binding with ECM components. They play a major role in adhesion, growth, migration and differentiation. These proteins are basically of two types, like – soluble (produced by liver hepatocytes) and insoluble (produced by various cells).

These proteins basically exist as a dimer (two nearly identical monomers, with same coding gene) linked together via a pair of disulphide bonds. Each chain has 5-6 domains and contains more or less 2500 amino acids. Each domain has their own specification. The domains are – 1) Fibrin binding domain 2) Heparin binding domain 3) Conserved **R-G-D** sequences in the Cell binding domain 4) Collagen binding domain 5) Heparin and fibrin binding domain.

Each chain consists of three types of repeating units, called FN repeats:

1) FN 1: contains 12 repeats, about 40 amino acid residues and 2 disulphide bonds.
2) FN 2: contains 2 repeats, about 60 amino acid residues and 2 disulphide bonds(interchain).
3) FN 3: contains 15 repeats, about 90 amino acids and without any disulphide bonds.

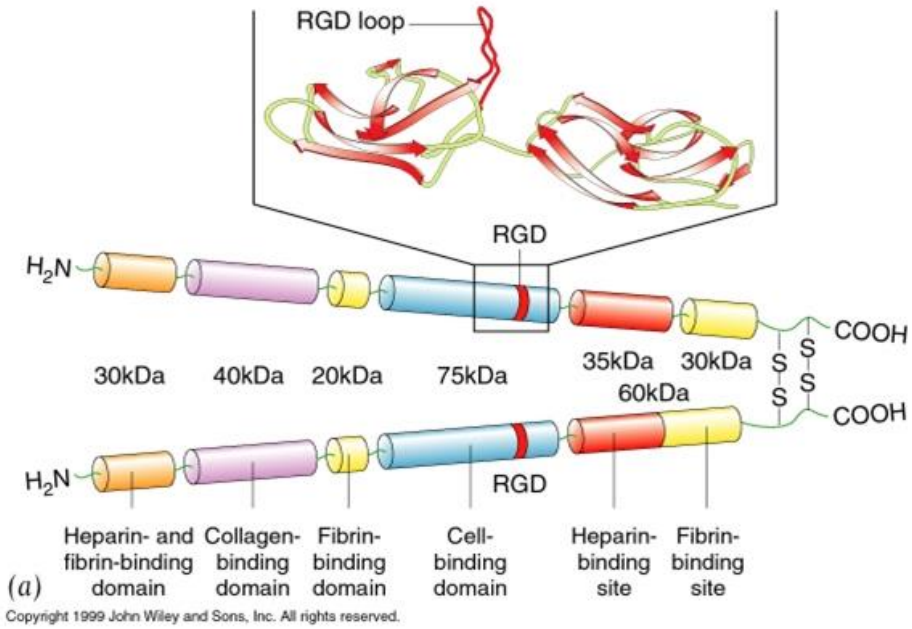


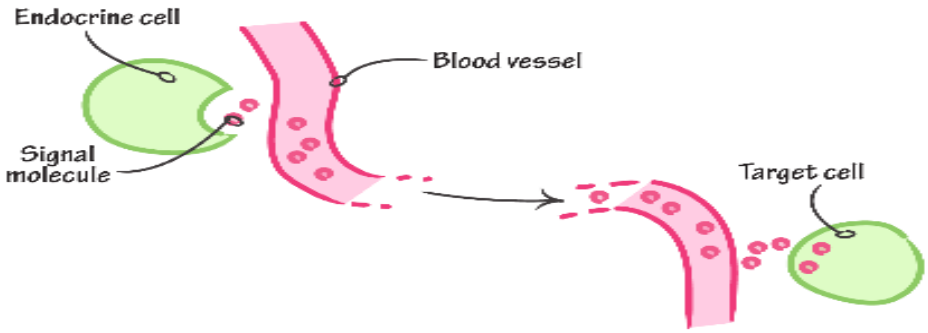
Fig.: Image of fibronectin

CELL SIGNALLING

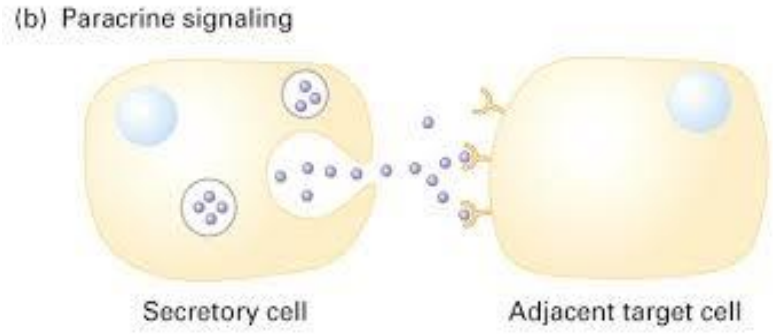
Every cell has a common property to give response against a stimulus or a signal. May be these signals are intracellular or may be extracellular. Cell signalling is a procedure through which an extra cellular signalling molecule activates a membrane receptor, that in turn alters intracellular molecules to generate a response.

Now the signalling machinery can be of different types:

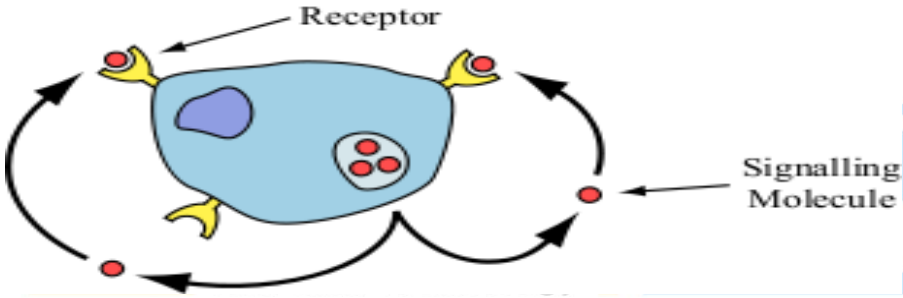
1) *Endocrine signalling*: In this case signalling molecule act on a target cell, distantly located from their site of synthesis and it occurs through the blood stream. Hormones basically act through this procedure.



2) *Paracrine Signalling*: In this case the signalling molecules released by a cell can affect another cell only when the target cell is in a close proximity. Neurotransmitters are the important examples of this.

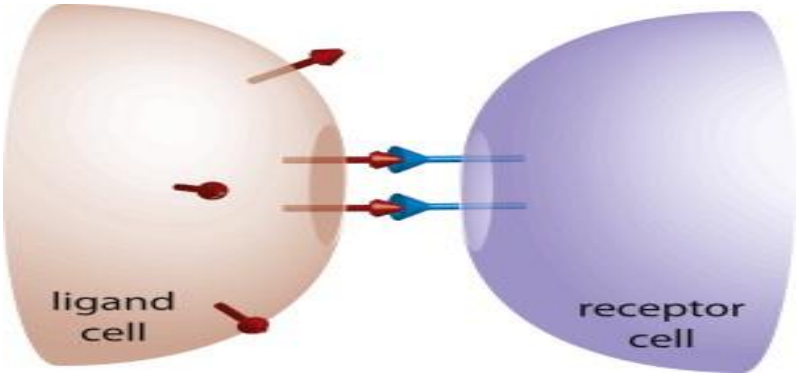


3) *Autocrine Signalling*: In this process the signalling molecule affect the same cell, producing it. Example- interleukin 1 acts on its producer monocyte.



4) *Juxtacrine Signalling*: This process basically requires a physical contact between two cells. Signalling molecules do not defuse out from the producing cell though it passes through the physical contact region. Example- Notch and Delta signalling.

Concept of Signalling Molecules



These are the molecules mainly responsible for transmitting the signal in between the cell and throughout the body. They can vary in size, shape and function. We can divide the molecules in two broad catagories:

1) *Lipophilic molecules*: They can diffuse across the plasma membrane and interact with the intracellular receptors, sometimes nuclear receptors too.
   Any kind of steroid hormones are within the examples.
2) *Hydrophilic molecules*: They basically bind to the cell surface receptors first and then generate a signal transduction pathway for functioning.
   Examples- ACTH, CCK, insulin, glucagon, gastrin, secretin, epinephrine, nor epinephrine etc.

In spite of these there are some steroid like hormones (example- α ecdysone, act as a steroidal prohormone-insect moulting hormone, secreted from the parathoracic glands) and some non-steroid like hormones (example- T4 and Retinoic acid).

Concept of Receptors

A signalling molecule will not be able to work at its target region without binding at a proper receptor. Each molecule has their specified receptors. There are also some receptors which are structurally similar to our known receptors but without any ligand (signalling molecule), they are known as Orphan Receptors (nuclear receptor superfamily). Receptors are basically protein or sometimes glycoprotein, located mainly at the surface of the target cell (plasma membrane) or cytosol or in nucleus. There are mainly two kinds of receptors, as follows:

Intracellular Receptors: They are located either in the cytosol or in the nucleus. Thyroid- steroid hormones and some intracrine peptide hormones, transcription factors use the intracellular receptors. All the intracellular (nuclear) receptors have a common structural similarity.

They have a N terminal and a C terminal end. From N to C they are divided into six structural and functional domains A to F. The A/B domain is reserved for AF1 or activation function 1, which act as a ligand independent transcriptional activator. Then comes the C domain or the DNA binding domain (DBD)- it consists of two zinc finger motifs (central zinc coordinates with four positioned cystein molecules). Then the D domain or Hinge region, act as a connector or linker. Then the E domain or ligand binding domain (LBD), which contains the AF2 region which helps to recruit transcriptional coactivator- the E domain is also responsible for homo and hetero dimerization of many nuclear receptors. Finally, the F domain, which is present with unclear functionalities.

Cell Surface Receptors**:** Mainly the hydrophilic signalling molecules can act through binding with cell surface receptors. These receptors basically act as a signal transducer to produce intracellular messengers. They are of three types mainly: 1) GPCR 2) Ion channel linked receptors 3) Enzyme linked receptors.

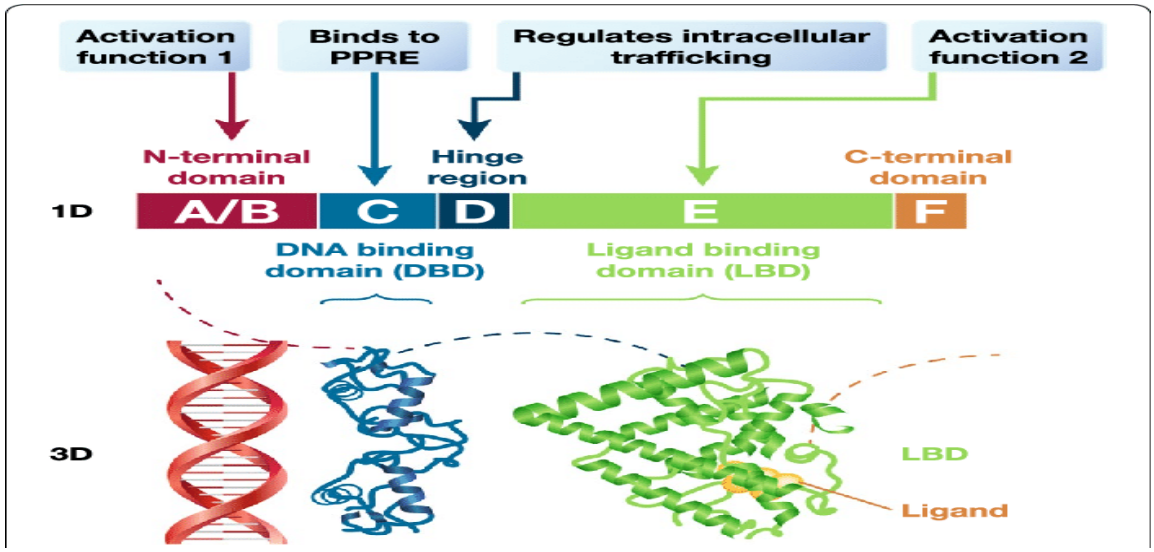


Fig.: Image of nuclear receptor

GPCR

Introduction: GPCR means G- Protein Coupled Receptor, present in all eukaryotes. GPCR includes smell, taste, light and pheromone sensing receptors mainly. It is the largest family of cell surface receptors which basically functions via G protein. Now the G proteins are Guanosine nucleotide binding protein. G proteins are basically two types, on basis of their subunits- trimeric (α, β, and γ) and monomeric (α/ βγ). G protein subunits remains in a hanging state (α hangs alone and βattaches with the hanging γ) with from the phospholipid inner leaflet, followed by a special conserved sequence **K-G-X-X-X-X-X-C**, where X is any amino acid except proline.

Structure: GPCR is a seven transmembrane protein (α helices) also called the Serpentine receptors, with N and C terminal, shows conformational change (outward movement of 5^{th} and 6^{th} TM helix) upon the ligand binding. The 7 domains of it are connected one by one through 3 EL (extracellular loops) and 3 IL (intracellular loops). The structure forms a cavity in the plasma membrane, serves as a ligand binding domain which is often covered by EL 2. The Most common structural theme of stability of GPCR is the palmitoylation of cystein residue via adding hydrophobic acyl groups through a covalent modification in lipid rafts. GPCR can be classified into 6 catagories based on sequence homology and functional similarities: class A, class B, class C, class D, class E, class F.

Mechanism of action:

When the ligand comes and binds to its specific site, the pre described conformational change occurs and immediately after it the GTPase activity of α subunit become on. The α subunit remains in its inactive state or GDP binding state but following the conformational change GTP get bound with the α subunit (with the help of GEF, guanine exchange factor) and make it

active.When the α subunit become activated it changes its position, get detached from the inner leaflet and attached with an effector molecule and enhance the effector molecule till the second messenger generates. When the generation of second messenger occurs, GAPs (GTPase activating proteins) are activated and promote GTP hydrolysis from the α subunit. Now GDP is bound with the subunit, which makes it inactive again and the energy released in this case, helps to slide back the subunit in its previous position. A specific system called RGS or regulator of g protein signalling controls the activity of GAP and GEF, thus regulate the entire process.

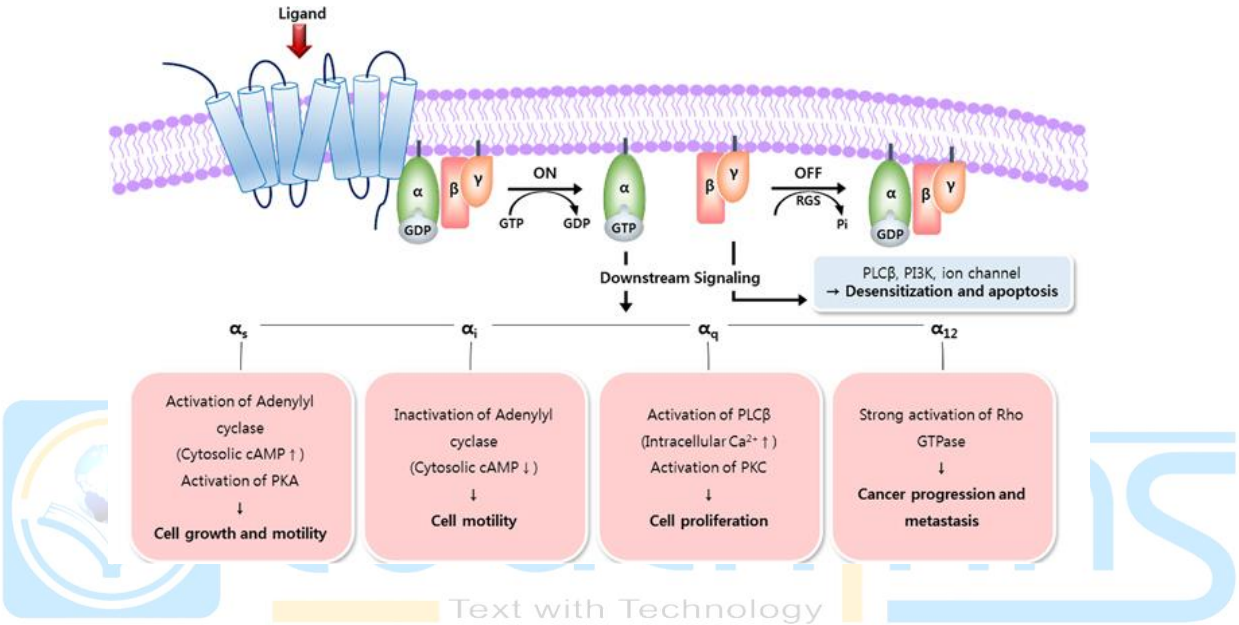
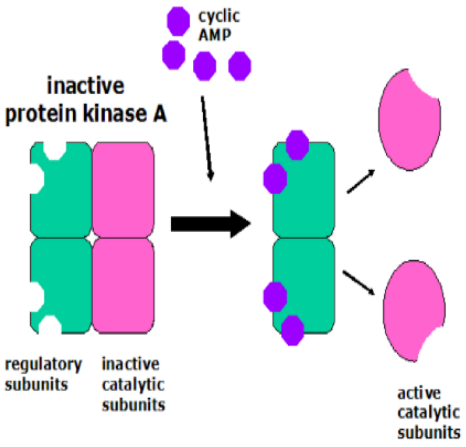


Fig.: Image of a common GPCR signalling pathway

SECOND MESSENGERS

There are a lot of second messenger types, involved in the signal transduction machinery. Among them few are very important, such as:



cAMP and PKA –

cAMP means cyclic adenosine monophosphate, the most important class of second messengers. They help in the opening of many ion channels in the membrane of different cells. But their main function is to activate protein kinase A or PKA. PKA has 2 catalytic subunits and two regulatory subunits. In the regulatory region there are cyclic AMP binding sites. cAMP binds their separate the regulatory units from the catalytic units. The regulatory units have NLS function, through which they

entered in the nucleus and promote CREB (cAMP response element binding protein) activation, which in turn promotes gene transcription. Protein kinases are also different types like PKB, PKC etc.

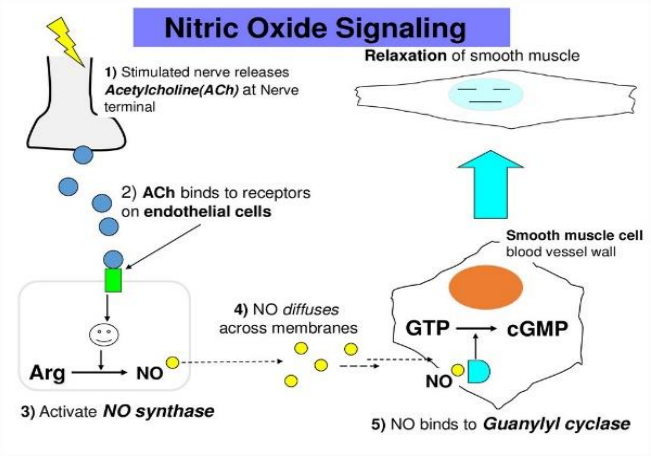
PLC -

PLC or phospho-lipase C is called the second most important second messenger class. Many G proteins exert their effects through PLC. There are different kind of PLCs – PLC β, PLC γ, PLC Ɛ, PLC δ etc. The naming of these are done basically according to the bond that it cleaves on a glycerophospholipid. It shows its main function in IP3 DAG pathway (discussed later).

CALCIUM-

Calcium is also one of the most important classes. It always functions in a combination form, with a highly conserved calcium ion binding protein Calmodulin. The Calcium-calmodulin complex basically acts through two pathways- via direct pathway that acts on target molecule and via the indirect pathway it acts on specific kinases (special S/T kinases, called CAM kinases).

NITRIC OXIDE-



The synthesis of nitric oxide is a very regulatory pathway, controlled by GPCR receptor signalling. Upon binding of ligand (acetyl choline mainly) the receptor gets activated and the GTPase activity of the α subunit becomes on thus activates the phospholipase cβ (PLCβ) from its inactive state. Active PLCβ then cleaves the PIP2 into two parts IP3 and DAG. This IP3 promotes the formation of calcium-calmodulin complex and this calcium calmodulin complex acts as the positive stimulator of NO synthase enzyme. In urea cycle, the NO synthase enzyme promotes the formation of NO and citrulin from arginine. The newly formed NO then diffuses out of the endothelial cell and act on the vascular smooth muscle or VSM. In the VSM, it basically activates the guanylate cyclase (GC), which in turn promotes the formation of cGMP from the GTP. cGMP then activates the PKG, which helps in the vasodialation and thus maintain blood pressure. This is also the functional mechanism of Viagra.

ION CHANNEL LINKED RECEPTOR

Ion channel linked receptors are called the ligand ion gated channels or Ionotropic receptors. These receptors are multipass transmembrane proteins mainly present in the sensory cells (neurons, myocytes etc). They are gated, and they may be open or closed, depending on the receptor binding of its specific ligands or by a change in the transmembrane electrical potential.

Example: In case of chemical synapse, electric signals are converted into chemical signals and they are further converted into electrical signals. When the action potential is generated at

the presynaptic neuron and the AP travels through it, ultimately come to the end part of neuron, then some voltage gated calcium channels are opened at that position. The opening of these channels causes influx of calcium inside the neuronal cell. And the calcium inserted, helps in the fusion of neurotransmitter contained synaptic vesicles. So, neurotransmitters are released at the synapse position and at the surface of post synaptic neuron there are a lot of neurotransmitter gated ion channels. Now depending upon the types of neurotransmitter, it will be decided that whether the signal effect as a n excitatory (opening of sodium channel-depolarization) or an inhibitory (opening of potassium channel – hyperpolarization).

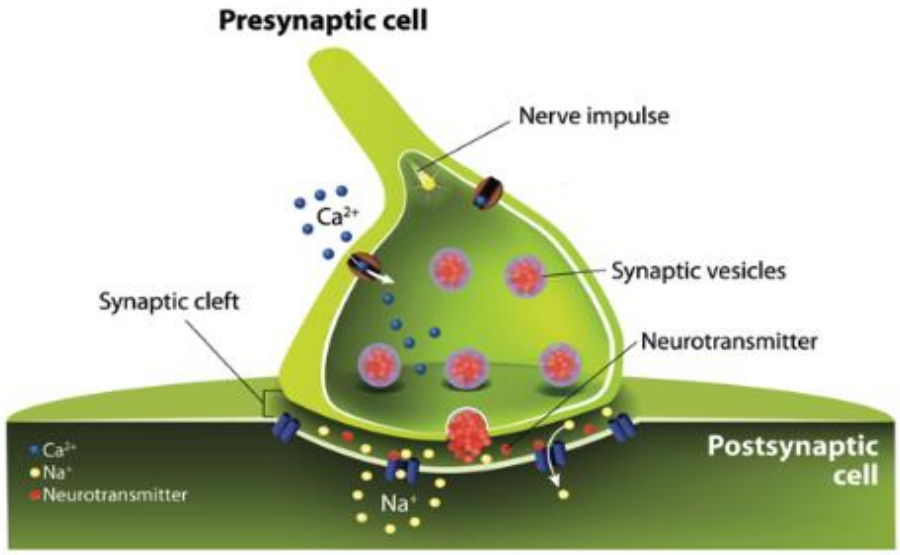


Fig.: Image of an ion channel linked receptor with reference to synaptic transmission

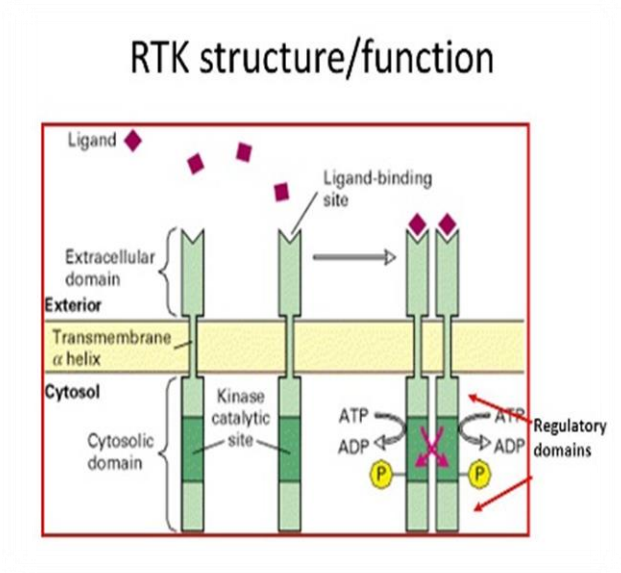
ENZYME LINKED RECEPTORS

These are also known as catalytic receptors and are mainly transmembrane receptors. These are the second most important type of cell surface receptors, where the extracellular ligands bind and causes enzymatic activity inside the cell. These have two domains mainly, an extracellular and an intracellular, which has the catalytic activity.

Example- 1) RTK or receptor tyrosine kinase 2) Tyrosine kinase associated receptors 3) Receptor serine threonine kinase. RTK is one of the importants.

RECEPTOR TYROSINE KINASES

They are the large multigene family of transmembrane cell surface receptors. They have a high affinity towards their ligands. The list of ligands of the RTK family are vast and of different catagories like- EGF, CSF, TGFα, PDGF, FGF etc. All the RTKs comprises a common structural similarity, they contain an extracellular ligand binding domain and an intracellular domain with a tyrosine kinase activity. Most of the RTKs show a receptor dimerization property upon the binding of ligand. There are many pathways for conducting the function of RTK, among them the importants are:



1) Ras- MAP kinase pathway
2) IP3-DAG pathway
3) PI3 kinase pathway

Ras-MAP Kinase Pathway

Ras Map kinase pathway is one of the best examples of receptor tyrosine kinase family and basically acts through activating a lot of protein components and finally enters into the nucleus to promote a lot of functions like cell division, differentiation etc.

Mechanism: Map kinase basically means Mitogen Activating Protein kinase (MAPK). The pathway begins upon binding of various kind of ligands, and follows a cascade mechanism, among them the one of the importants is Insulin. The pathway is described as follows under some noteworthy points:

1) The structure of insulin hormone receptor is α2β2, present in the lipid layer. The alpha part is extracellular and the beta part is intracellular. This structure is not an active structure because an inhibitory sequence in the beta subunit always the activity of the structure.
2) When the ligand binds to its receptor the inhibitory sequence removed thus creates a conformational change and the active sites of the structure exposed.
3) Now the beta subunit has its own phosphorylation property called the autophosphorylation. It is noticed that the phosphorylation follows a conserved sequence of **N-X-Y-P**. When the phosphorylation occurs, the structure become active.
4) When the receptor activation occurs, a special sequence called IRS or insulin receptor binding sequence attached with the receptor, and then this is also phosphorylated by the activated β subunit.

5) When the IRS is phosphorylated, another protein called Grb-2 or growth receptor binding protein 2 attached with it. This Grb-2 is basically an adaptor protein which contains a special domain called SH2 domain (or Shark Homology 2 domain) through which it binds with the Grb-2.
6) When the Grb-2 get attached with it, a special kind of protein called SOS or Son of Sevenless protein bind with the Grb-2 (in the SH3 domain). This SOS has special kind of GEF activity.
7) A protein named as Ras or rapidly accelerating sequence is present in the cytosol and remains in its inactive state (GDP binding state). Ras has a GTPase activity- when SOS binds with the Grb-2, the GTPase activity of Ras becomes on and GTP binds with it and make it active.
8) Activated Ras also activate the next protein called Raf, or rapidly accelerated fibrosarcoma by phosphorylation in S/T residue.
9) Activated Ras further activates the next protein called MEK or MAPKK through phosphorylation in its Y/T residue.
10) Activated MEK further phosphorylate the next and last cytosolic protein called ERK or extracellular regulatory kinase or classical MAP kinase or MAPKKK, in its S/T residue. This ERK has NLS sequence for nuclear localization, through which it can enter in to the nucleus.
11) In the nucleus, there are two more factors responsible for the transcription activation, SRF (serum response factor) and ELK-1 (ETS domain regulatory kinase). ERK basically does two things- phosphorylate ELK-1 and promote the SRF to bind with the ELK-1.
12) Now this ELK-1 and SRF complex act as the transcription factor and thus promoting cell division, cell differentiation, survival, EC attachments etc.

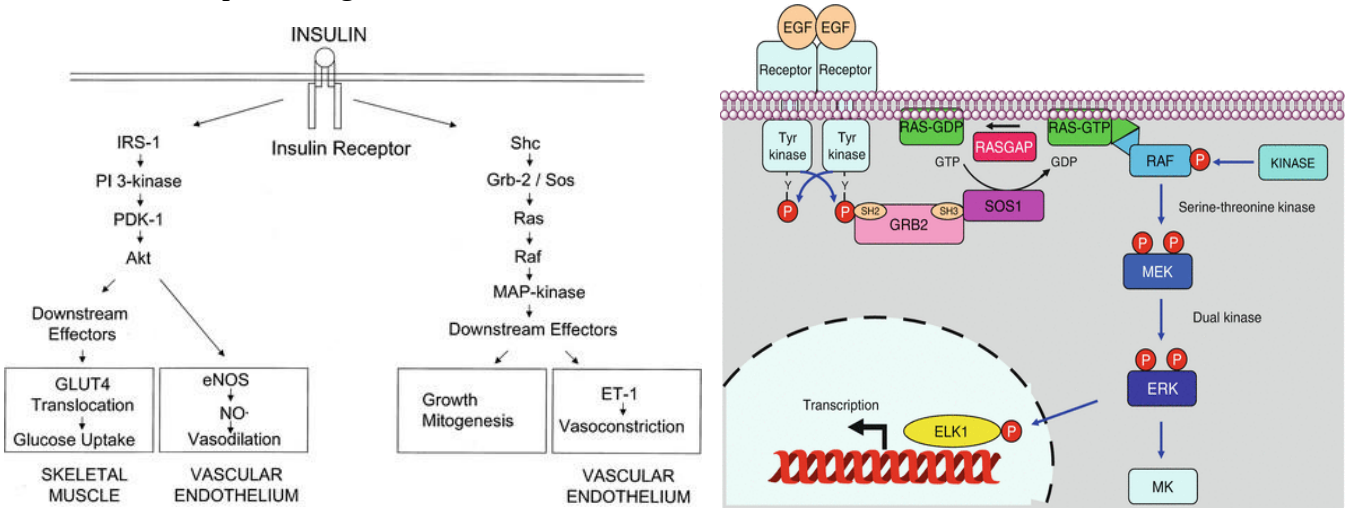
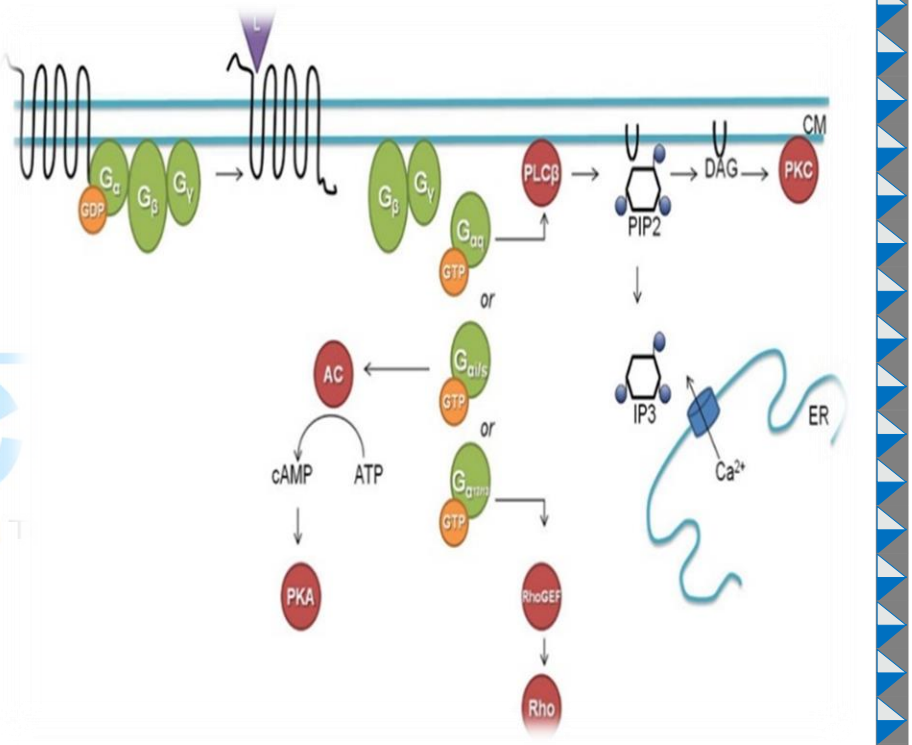


Fig.: Image of MAP kinase pathway through two ligands- Insulin and EGF

IP3-DAG PATHWAY

One of the important widespread pathways of intracellular cell signalling. It is also based on the use of second messenger PLC (phospholipase C), which acts on the membrane phospholipid of inner layer PIP2 (phosphatidyl inositol 4,5 bisphosphate). It basically acts through GPCR receptor.

Mechanism: When the ligands come to bind with the receptor of GPCR, the Gq type of heterotrimeric G protein (α subunit) become activated. Thus, induce the transfer of PLC β inactive state to its active conformation (in case of RTK receptor involvement PLC γ activates). Active PLC β cleaves the PIP2 on the glycerol side of phosphodiester bond and breaks into two components IP3 (inositol tri phosphate, formed from an intermediate inositol 1,2 cyclic phosphor di ester) and DAG (di acyl glycerol). Now the PLC A2 acts on the DAG and promote the synthesis of phosphatidic acid. On the other hands the IP3 controls the intracellular calcium concentration. IP3 promotes the calcium channel opening on SER membrane. That means it can sense the intracellular calcium level. When the intracellular calcium level falls down at a certain level, IP3 converts into IP4 by the enzyme IP3-3 kinase and this IP4 helps in opening of calcium channels in the plasma membrane thus promotes the calcium entry into the cytosol.



PI3 KINASE PATHWAY

PI-3 kinase pathway is known as phospho-inositide pathway, is a family of intracellular lipid kinase family. It occurs through the involvement of RTK family receptors. Various ligands can bind to promote the cellular functioning. When the ligands bind to the receptor, the intracellular domain of the receptor undergoes phosphorylation (mainly at 85 and 110 position). When the receptor phosphorylation occurs PI3 binds with it and catalyses the formation of PI-3,4,5. This PI-3,4,5 binds with inactive PKB or protein kinase B. The PKB has two domains – S/T kinase domain and PH or plectrin homology domain. So, PI-3,4,5 binds with PKB, means the concentration of PKB increases in the plasma membrane. Now the PH domain helps in a kinase recruitment in the plasma membrane, called PDK-1 or phosphoinositide dependent kinase-1.

This PDK-1 does a phosphorylation in the PKB, and make it active. When the PKB become active, it dissociates from the plasma membrane and phosphorylated many target proteins and finally helps in cell growth, cell proliferation, motility, cellular trafficking etc.
(The protein kinase B or PKB has another name called AKT, which denotes the Thymoma cell line of AKR mice (albino mice used in cancer research; Thymoma is tumour of epithelial cell)

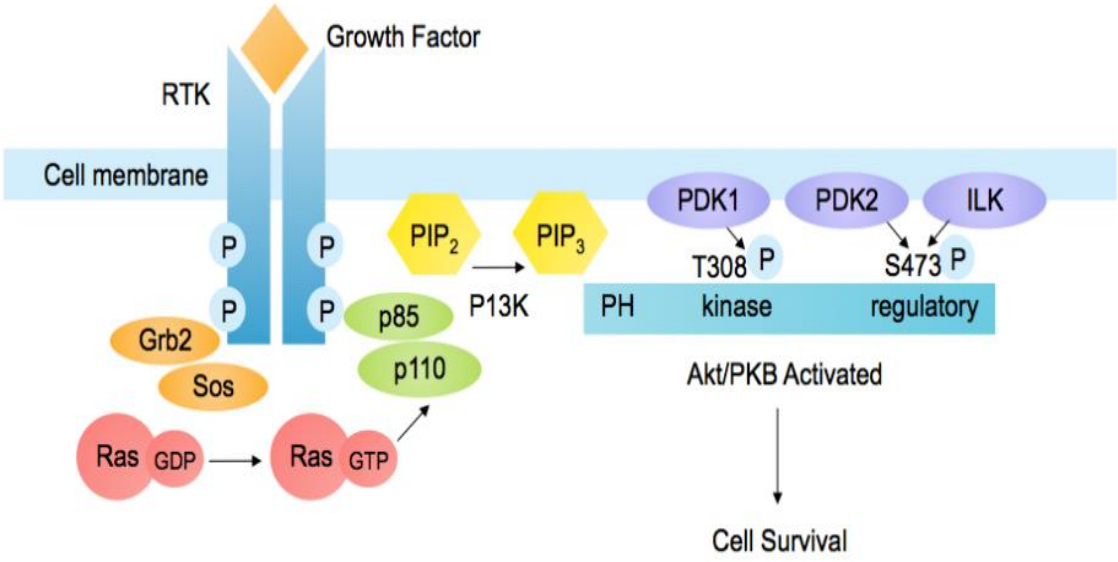


Fig.: PI3 kinase pathway

JAK STAT PATHWAY

This is another important pathway through which signal from the out, goes towards nucleus and resulting in DNA transcription and expression of genes thus involved in the immunity, proliferation, differentiation, apoptosis and oncogenesis.
The word JAK means Janus (dual nature) Kinase, and another term STAT means Signal Transducer and Activator of Transcription. This pathway also works through a cascade mechanism.
Mechanism: The binding of the different kinds of ligands (cytokines, interleukins, interferons, growth factors etc.) activates the receptor JAK and increase their kinase activity. Activated JAKs then phosphorylate (autophosphorylation) at its tyrosine residue (Y1138) and creating a binding site for STAT. STAT is a cytosolic protein can act as a transcription factor. With N terminal and C terminal STAT also contains a SH2 domain. Through this SH2 domain they bind with phosphorylated JAK receptor, where they are also phosphorylated by the JAKs and become activated. Activated STAT can therefore form dimer. And STAT dimer contains special NLS sequences through which they can enter into the nucleus.

Inhibitors of the pathway: there are two kind of inhibitors cytosolic and nuclear. SOCS or suppressor of cytokine signalling family proteins can inhibit the phosphorylation of STAT (example- protein tyrosine phosphatases). Another inhibitor called, PIAS or protein inhibitor of activated STAT, acts in the nuclear level and negatively regulate the STAT through special mechanism.

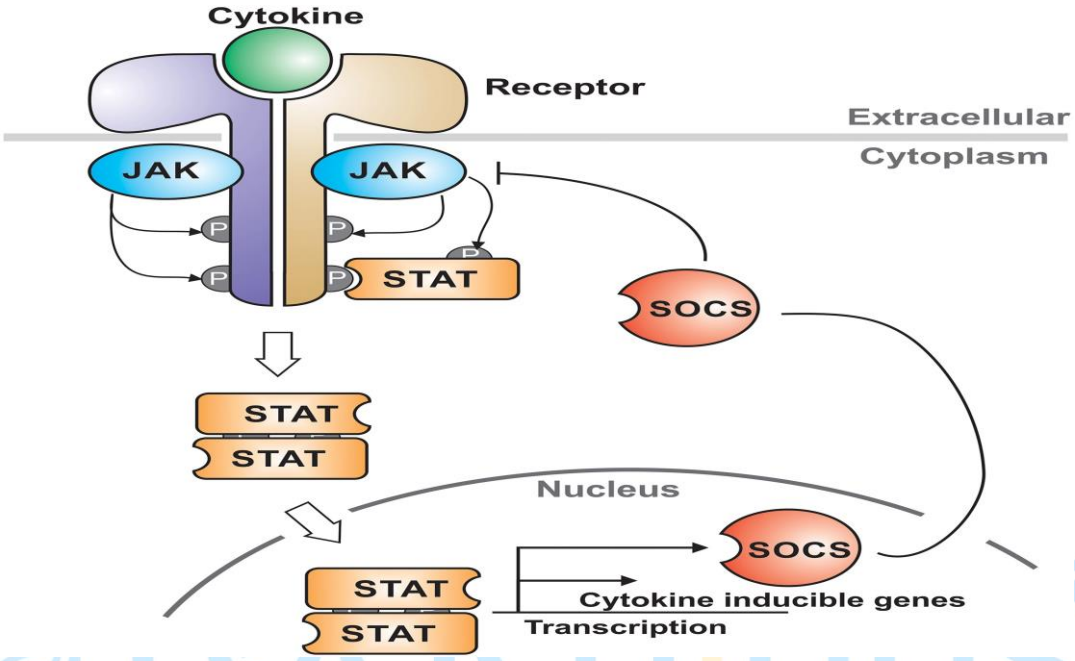


Fig.: Image of JAK-STAT signalling

TWO COMPONENT SIGNALLING

Two component system acts as a basic stimulus response mechanism, in various environmental condition. It is seen in all domains of life specially in bacteria (gram -ve, cyanobacteria). But suspiciously it is absent in the animals.

The name two component system is named due to the signalling receptor of this pathway, which is a special histidine kinase receptor. This receptor can show two activities, one is kinase activity and the another is phosphatase activity. Histidine kinase is a homodimer (HWE and HIS KA2 are the non-homodimer histidine kinase examples) with special domains, one is- N terminal histidine phospho-transfer domain and the another is C terminal ATP binding domain. When specific ligand comes and bind to its receptor, the N terminal domain become activated and which promotes an autophosphorylation of the receptor thus made the receptor active. When the receptor becomes active, the N terminal phosphor-transfer domain transfer an ATP to a histidine residue of the receptor. Following this a sensor protein becomes activated and this protein transfers the phosphate group of histidine to an aspartate residue, via histidine phospho-transferase enzyme. This aspartate residue is one of the important

active site amino acid of the Response Regulator's receiver domain. Now the response regulator (RR) is one of the important regulators of the pathway, with two domains – receiver domain and effector output domain. When the reciever's aspartate residue is phosphorylated, the second domain become active, and involves in the DNA binding, thus promotes the gene stimulation and helps in different functions like- quorum sensing, osmolarity changing, operon regulation, chemo attractivity etc.

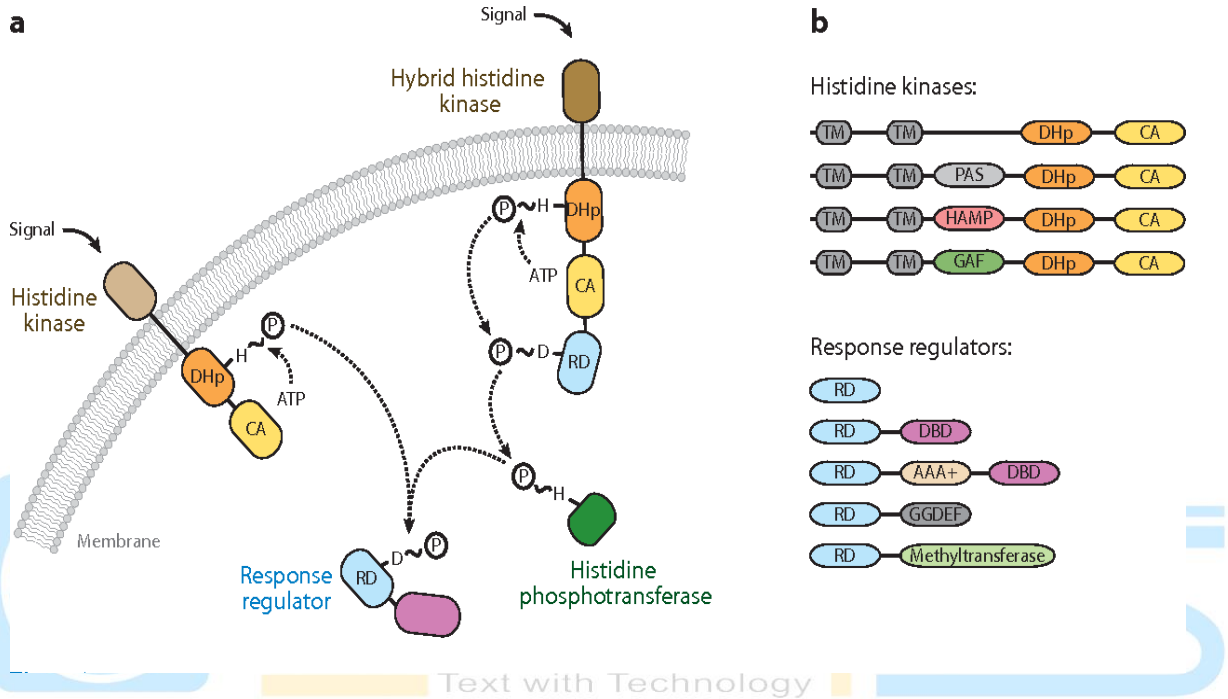
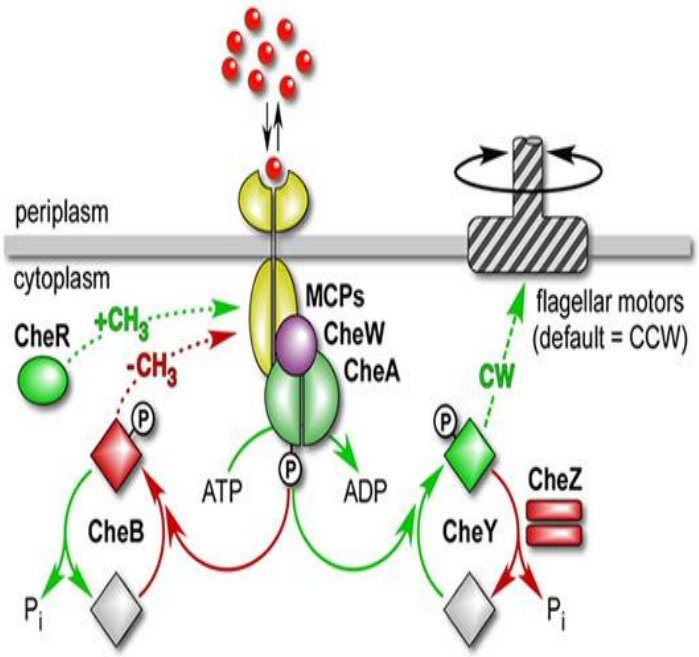


Fig.: Image of bacterial two-component signalling

CHEMOTAXIS

Chemotaxis is a special type of movement which can be seen, as a response against an extracellular chemical signal concentration. This activity is seen in bacteria and other single or multicellular organisms. The chemotactic movement occurs through specific organs like flagellum. Mot A, Mot B, Flig G (one of the Flig family proteins) etc. are involved in the movement of the flagellum. Now according to the type of chemical response, the flagellar movement occurs, for example, the applied chemical stimulus can be attractant or can be repellent. So, according to the type of stimulus, movement will be changed – in case of attractant stimulus the movement of the flagella will anti-clock wise, allow the organism to run towards it: and in case of repellent stimulus the flagellar movement occurs in a clockwise direction which causes the organism to be tumbled.

Mechanism: Suppose the concentration of a repellent increases in a media. It causes the positive activity in the transmembrane receptors. When the receptors become active, they bind with an adaptor protein (Che-W) and with a sensor protein (Che A) which is histidine kinase in nature. So, Che-A is autophosphorylated in its histidine residue. Then the phosphate group of it is transferred to the aspartate residue of a response regulatory element, Che-Y and make it phosphorylated (Che-Y-P). when it becomes phosphorylated it dissociates from the receptor, diffuses through cytosol. It binds to the flagellar motor causes it to rotate clockwise thus made it tumble. Here one notable point is that, Che-Y remains in phosphorylated state for few seconds only, after that another important protein Che-Z dephosphorylate it.



In presence of attractant the receptor inactivation occurs and opposite effects do the anticlockwise movement of it.

Another important protein, Che-R promotes receptor methylation during adaptation (the response towards a stimulus is always transient, even if the higher concentration is maintained for a long time, bacteria will desensitize or adapt) thus they are also called MCP or methyl accepting chemotaxis protein.

QUORUM SENSING

This is a special types of communication mechanism, commonly seen in case of bacteria. Through the machinery they communicate themselves via secreting of signalling molecules, and thus assess the density of population. This procedure is also functional in case of genomic level regulation and also in behaviour.

These signalling molecules are also called autoinducers. Generally, gram negative bacteria use N-acetyl homoserine lactones (AHL) and gram-positive bacteria use oligo peptides as the autoinducers. When these autoinducers bind in specific receptors, they promote the transcription of a set of genes including those- producing inducers. Quorum sensing covers a lot of physiological activities like symbiosis, virulence, competence, conjugation, motility etc. A gene called lux-I gene encodes the AHL synthase, which promotes the formation of AHL. It is a process of autoregulation. So, we can say that AHL level will increase in cell if the transcription of the gene occurs in a large amount. A transcriptional activator also included in this machinery, which is called lux-R. This lux-R basically binds with the AHL. This AHL

diffuses out from the cell and directly communicate with the environment. So we can say that the concentration of AHL is basically depends upon the cell density.

From the previous talk we know that increasing level of AHL promotes its own gene expression along with other genes- so, when the concentration of it increases, it flow back into the cell and regulate other gene expression which are important for causing bioluminescence. On other hand we can say that this bioluminescence cannot be seen when the cell is alone, it will only be seen in case of a large cell density.

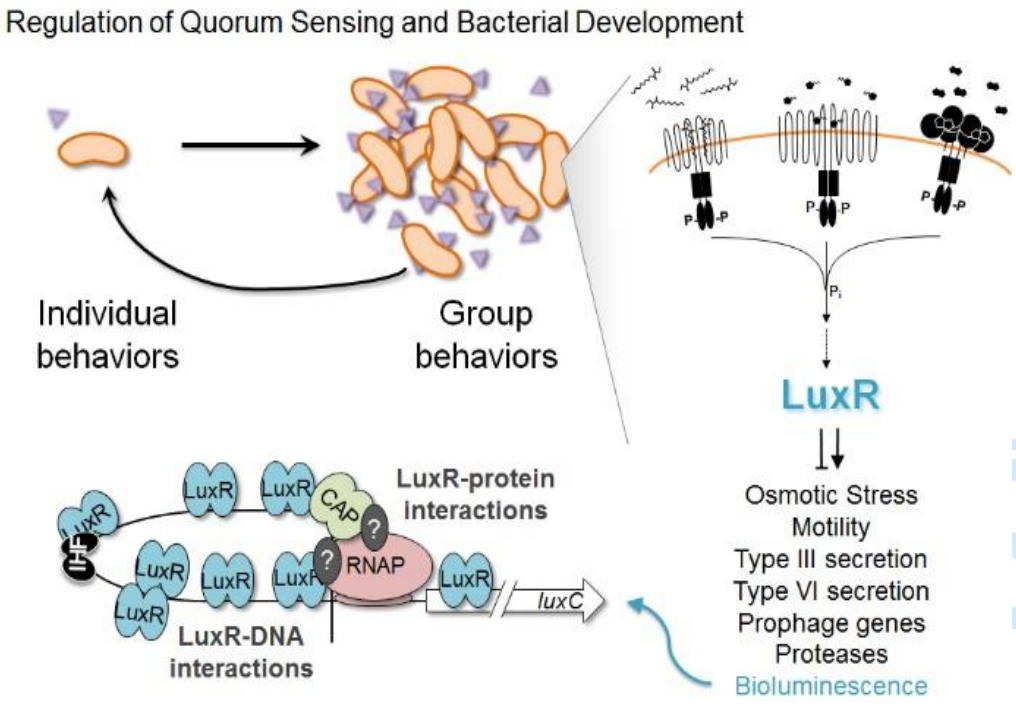


Fig.: Bacterial quorum sensing

CANCER

Cancer is a class of disease characterized by uncontrolled cell growth or when cells of the body at a particular site in the body start to grow out of control. Cancerous cell characteristically different from normal cells. Instead of controlled dying (apoptosis) cancerous cells may continue to grow and form new, abnormal cells in the body. Cancerous cell rapidly grow in the body and multiply themselves and form a lumps or masses of tissues that is known as **tumor.** Tumors can be of two type malignant tumor and benign tumor depending upon whether the tumor can spread throughout the body by invasion and metastasis.

Benign Tumor:- Benign tumors are typically slow growing and rarely they spread from one tissue to other. They contain a well-defined border so that they can be easily remove by surgical treatment. However benign tumor in brain harms an individual adversely. In other parts of body the presence of benign tumor is not so dangerous as compared to malignant tumor. Benign tumors in the area of brain that control respiratory functions may consider as malignant tumor. Cells of this kind of tumors are closely like normal cells and may as like normal cells.

Malignant Tumor: By definition malignant tumor is the **Cancer** that means malignant type tumor can be spread from one part of the body to another part of the body. Malignant tumors are life threatening type tumor.

Cancerous cells are also known as neoplastic cell and the tumor itself is known as neoplasm.

DIFFERENCES BETWEEN NORMAL CELLS AND TUMOR CELLS:

The body is made up of approximately 37.2 trillion human cells – so you can truly appreciate how many that is, here is the number written out in full, 37,200,000,000,000– These 'normal' cells act as the body's basic building blocks and possess specific characteristics that enable them to maintain correct functioning of tissues, organs, and organ systems. Normal cells:

- control their growth using external signals, meaning they only grow and divide when required,
- undergo programmed cell death (apoptosis) as part of normal development, to maintain tissue homeostasis, and in response to unrepairable damage,

'stick together' by maintaining selective adhesions that they progressively adjust which ensures they remain in their intended location,

- Differentiate into specialized cells with specific functions meaning they can adopt different physical characteristics despite having the same genome.

Cancer is a complex genetic disease that is caused by specific changes to the genes in one cell or group of cells. These changes disrupt normal cell function – specifically affecting how a cell grows and divides. In contrast to normal cells, cancer cells don't stop growing and dividing, this uncontrolled cell growth results in the formation of a tumor. Cancer cells have more genetic changes compared to normal cells, however not all changes cause cancer, they may be a result of it. The genetic changes that contribute to cancer usually affect three specific types of gene; proto-oncogenes, tumor suppressor genes, and DNA repair genes.

There are several differences characteristically found between normal cells and cancerous cells or neoplastic cells.

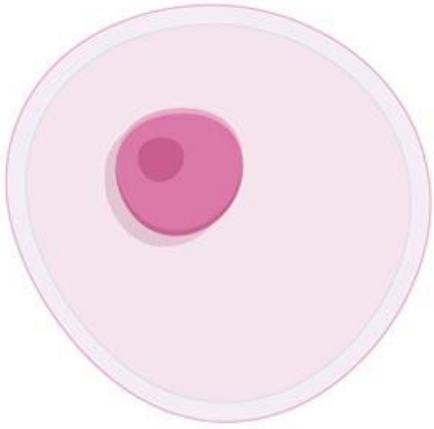
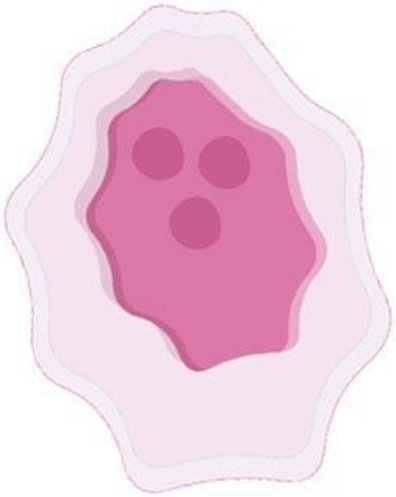
Cell Shape: Normal human cells come in many shapes and sizes – as they differentiate and adopt specialized functions their shape changes accordingly – for instance a red blood cell looks very different to a nerve cell. *Different* types of cells do not look alike, but, if you analyse cells of the *same* cell type they will look extremely similar, maintaining a uniform shape. For years researchers have been peering down microscopes, looking for distinct features that can help them determine the difference between a cancer cell and normal cell. Cancer cells are misshapen, and appear as a chaotic collection of cells, in an array of shapes and sizes. Researchers have been investigating the relationship between cancer cell shape and a patients' outlook, and whether cell shape may also help to distinguish between the different types of cancer.

Nucleus: In normal cells the nucleus has a smooth appearance and maintains a uniform, spheroid shape. Several structural components are involved in the_regulation of nuclear morphology. One of these structural components is the nuclear lamina. Cancer cell nuclei are frequently misshapen and bulges known as "blebs" can often be observed in cells' nuclear membranes. Research suggests that this 'blebbing' is caused by an imbalance in the proteins that constitute the nuclear lamina which leads to separation of the lamina fibers.

Chromatin: The fine, evenly distributed chromatin found in normal cells transforms into coarse, chromatin in cancer cells – aggregating into irregular clumps that vary in both size and shape.

Nucleolus: Tumor aggressiveness and clinical outcome can both be measured by observing the morphology of a cancer cell's nucleolus/ nucleoli. The nucleolus becomes increasingly enlarged and more irregular in cancer cells – cells can have multiple nucleoli within the nucleus.

Blood supply: Angiogenesis is defined as the development of new blood vessels that form from pre-existing vasculature. Angiogenesis is a vital process in normal cells that occurs during development, growth, and wound healing. However, it is also implicated in the growth of cancer, through the tumor's ability to secrete chemical signals that stimulate angiogenesis

	Normal Cell	Cancer Cell
		
Cell shape	Uniform	Irregular
Nucleus	Spheroid shape, single nucleus	Irregular shape, multi-nucleation common
Chromatin	Fine, evenly distributed	Coarse, aggregated
Nucleolus	Single, inconspicuous nucleolus	Multiple, enlarged nucleoli
Cytoplasm	Large cytoplasmic volume	Small cytoplasmic volume
Growth	Controlled, slow	Uncontrolled, rapid
Maturation	Mature into specialized cells	Remain immature and undifferentiated
Blood supply	Normal angiogenesis (occurs during development/ healing)	Tumor-induced angiogenesis
Oxygen	Favored (for aerobic respiration) but will undergo anaerobic respiration if required	Not required (thrive in hypoxic conditions), favour anaerobic respiration
Location	Remain in their intended location	Can spread to different locations in the body (metastasis)

The loss of cellular regulation on cell division of tumor cells is due to some genetic damages that is often accompanied by influences of tumor promoting chemicals that means carcinogens and some sometimes it is hormone and sometimes it is virus. The genes that the may be defined as proto oncogenes and tumor suppressor genes. Proto-oncogenes normally promote the cell growth they are changed into oncogenes by mutation that causes the gene to be excessively active in growth promotion. Where is tumor suppressor genes sustain or restrict the growth of cells so mutations that inactivate them allowed in appropriate cell divisions that means the onset work cancers or more specifically the onset of tumor.

Another class of gene known as caretaker genes are also often linked to cancer. Caretaker genes normally protect the integrity of the genome when they are in activity IT sales aqua additional mutation at an increased rate including mutations that damage growth control and lead to cancer.

cancer commonly results from mutations that arise during the lifetimes exposure to carcinogen that means carcinogens are that kind of material that influence proto-oncogene to become transform into oncogenes and this carcinogens also inactivate the tumor suppressor genes. Carcinogens include certain chemicals and ultraviolet radiations that are present in environment.

cancer causing mutations occurs mostly in somatic cell not in the germ-line cells, and somatic cell mutations are not passed onto the next generation. So that means cancer is not a genetic disease.

The mechanism of onset of Cancer may be referred to as oncogenesis or tumor genesis is an interplay between genetics and the environment. Many years may be required to obtain severe mutations that will ultimately cause the onset of cancer. So most of the cases found that the cancer found in the later stage of the life. Furthermore it has been shown that the cancer occurs most frequently after the age of reproduction.

The mutated cells acquire special ability to escape from normal epithelia and stimulate the growth of vasculature (angiogenesis) to obtain adequate oxygen for their metabolism. Gradually the clone of cells turned into tumor. In some cases (malignant tumor) cells from the primary tumor site migrate to new sites and form secondary tumor, this process is known as metastasis. Most of the death due to cancer are the result of invasive, fast growing metastasised tumors.

GENETIC BASIS OF CANCER:

Mutations in normal cells mediated by two broad classes of genes— proto-oncogenes (like ras) and tumor-suppressor genes (like APC)—play key roles in cancer induction. These genes encode many kinds of proteins that help control cell growth and proliferation. Virtually all human tumors have inactivating mutations in genes that normally act at various cell-cycle checkpoints to stop a cell's progress through the cell cycle if a previous step has occurred incorrectly or if DNA has been damaged. For example, most cancers have inactivating mutations in the genes coding for one or more proteins that normally restrict progression

through the G1 stage of the cell cycle. Likewise, a constitutively active Ras or other activated signal-transduction protein is found in several kinds of human tumor that have different origins. Thus malignancy and the intricate processes for controlling the cell cycle are two faces of the same coin. In the series of events leading to growth of a tumor, oncogenes combine with tumorsuppressor mutations to give rise to the full spectrum of tumor cell properties.

Genes control how your cells work by making proteins. The proteins have specific functions and act as messengers for the cell. Each gene must have the correct instructions for making its protein. This allows the protein to perform the correct function for the cell. All cancers begin when one or more genes in a cell mutate. A mutation is a change. It creates an abnormal protein. Or it may prevent a protein's formation. An abnormal protein provides different information than a normal protein. This can cause cells to multiply uncontrollably and become cancerous. Mutations happen often. A mutation may be beneficial, harmful, or neutral. This depends where in the gene the change occurs. Typically, the body corrects most mutations. A single mutation will likely not cause cancer. Usually, cancer occurs from multiple mutations over a lifetime. That is why cancer occurs more often in older people. They have had more opportunities for mutations to build up.

Tumor suppressor genes: These are protective genes. Normally, they limit cell growth by:

- Monitoring how quickly cells divide into new cells
- Repairing mismatched DNA
- Controlling when a cell dies

When a tumor suppressor gene mutates, cells grow uncontrollably. And they may eventually form a tumor.

Examples of tumor suppressor genes include *BRCA1*, *BRCA2*, and *p53* or *TP53*.

Germline mutations in *BRCA1* or *BRCA2* genes increase a woman's risk of developing **hereditary breast or ovarian cancers** and a man's risk of developing hereditary prostate or breast cancers. They also increase the risk of pancreatic cancer and melanoma in women and men.

The most commonly mutated gene in people with cancer is *p53* or *TP53*. More than 50% of cancers involve a missing or damaged *p53* gene. Most *p53* gene mutations are acquired. Germline *p53* mutations are rare, but patients who carry them are at a higher risk of developing many different types of cancer

Oncogenes: These turn a healthy cell into a cancerous cell. Mutations in these genes are not known to be inherited. Two common oncogenes are:

- HER2, a specialized protein that controls cancer growth and spread. It is found in some cancer cells. For example, breast and ovarian cancer cells.
- The *RAS* family of genes, which makes proteins involved in cell communication pathways, cell growth, and cell death.

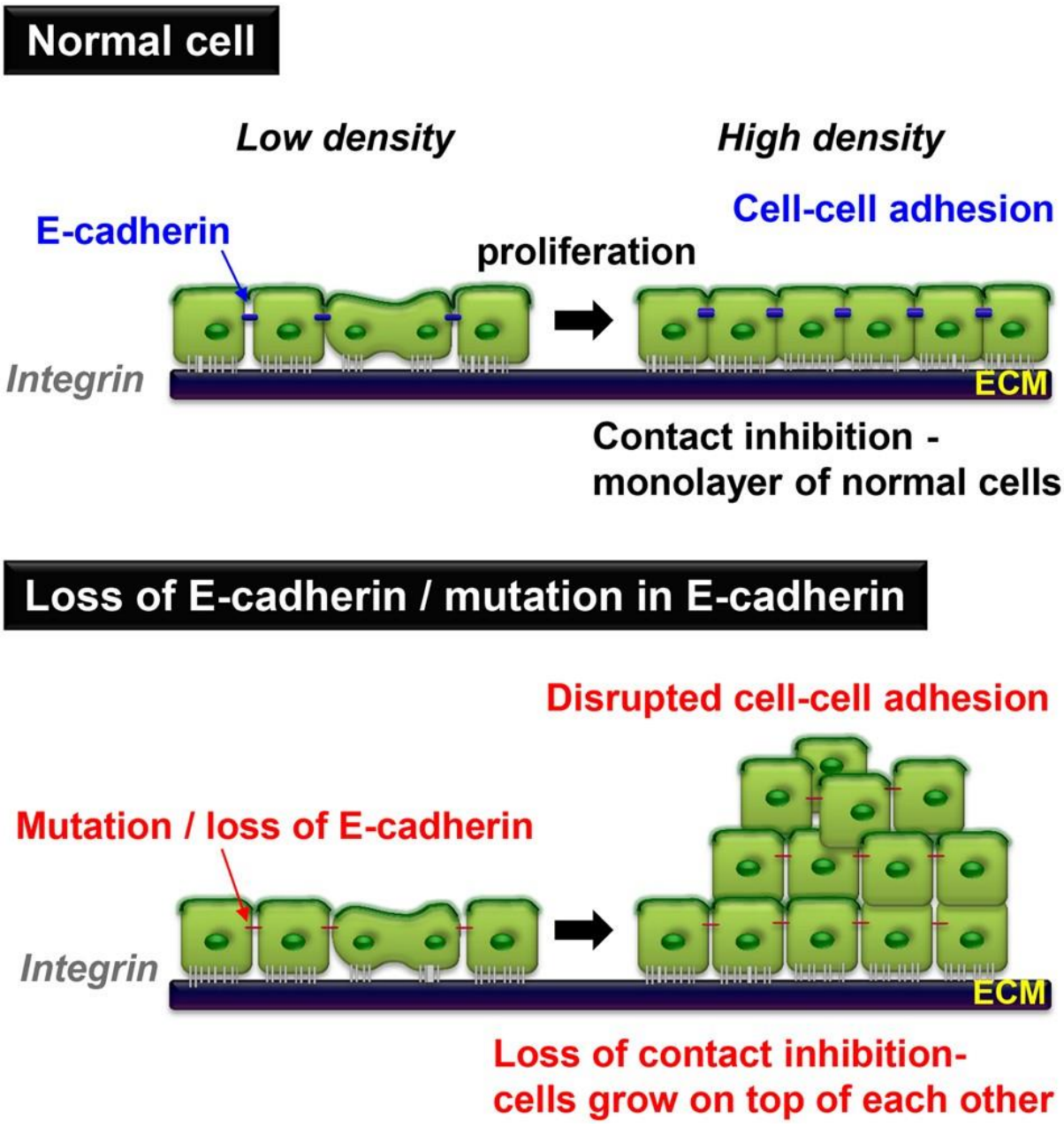
DNA repair Genes or Caretaker Genes: These fix mistakes made when DNA is copied. Many of them function as tumor suppressor genes. *BRCA1*, *BRCA2*, and *p53* are all DNA repair genes.

If a person has an error in a DNA repair gene, mistakes remain uncorrected. Then, the mistakes become mutations. These mutations may eventually lead to cancer, particularly mutations in tumor suppressor genes or oncogenes.

Mutations in DNA repair genes may be inherited or acquired. **Lynch syndrome** is an example of the inherited kind. *BRCA1*, *BRCA2*, and *p53* mutations and their associated syndromes are also inherited.

PROPERTIES OF CANCER CELL:

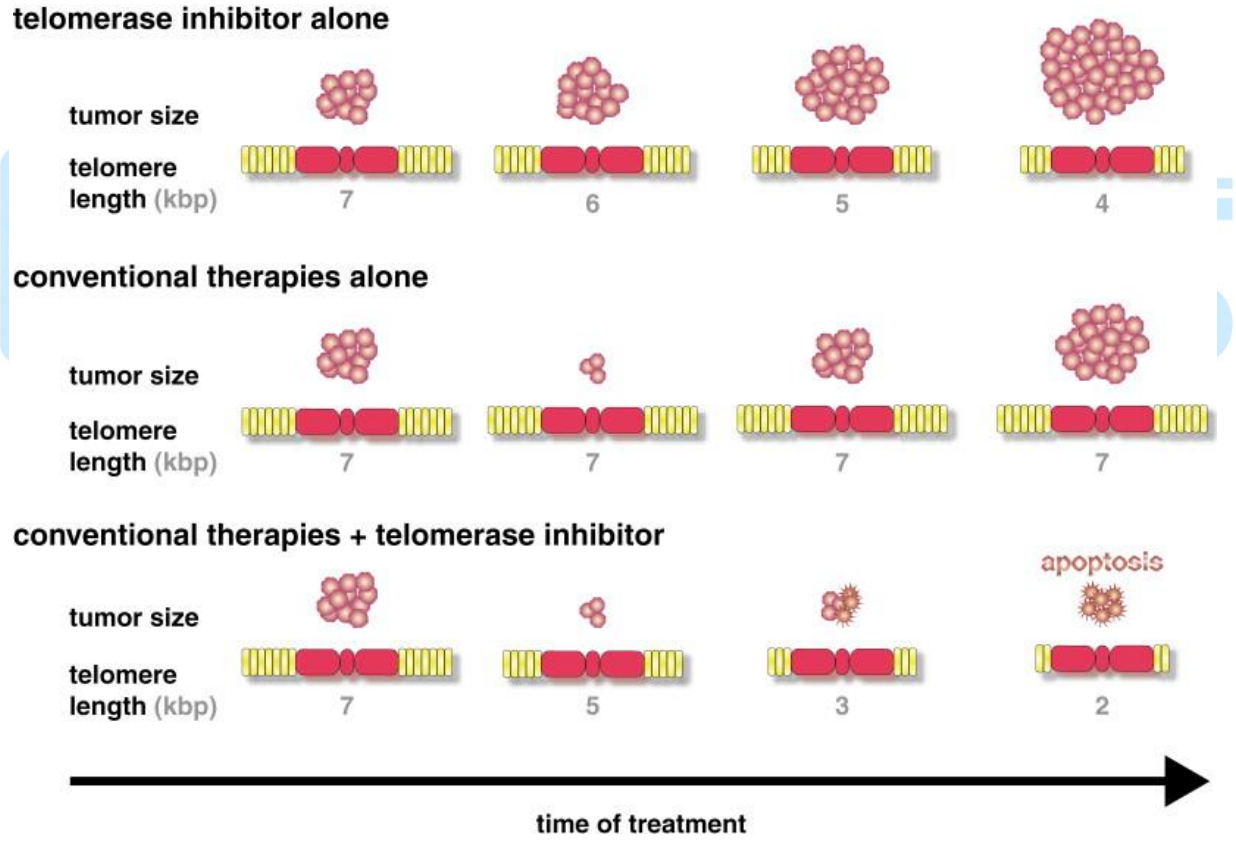
1. **Density dependent inhibition:** Normal cell divide until it reach a certain or finite density that means when normal reach their maximum capacity of the tissue it ceases its cell division or sometimes transferred G_0 or quiescent state. But cancer cell did not follow this type of regulation of cell division. They divide infinitely without depending upon the capacity of the tissue. And thus they form tumor or lumps of cell in the tissue.
2. **Contact Inhibition:** As like normal cells cancer cells show contact between adjacent cells through tight junction, gap junction to establish biological communication between cells. Normal cells cease their cell division and induce adjacent cells to stop further division after the tissue reach its maximum capacity by communicating between each other by means of several growth factor release. However cancer cells did not follow this type of cellular control thus proliferate in uncontrolled manner.



3. **Immortalization- Effect of Telomerase:** Telomeres are repeated component of DNA found in the end part of the chromosome. Telomerase is a eukaryotic enzyme that shows telomere protecting capacity. Normal cells do not produce telomerase (except rapidly growing cells in lungs, GI tract). In most of the cells telomeres become gradually shorter as the progress several cell division. After several division the telomere became so short that no further cell division can take place due to absence of appropriate length of telomere.

The TERC gene is responsible for production of telomerase enzyme. Telomerase prevent the shortening of telomeres by adding small repeated segments of DNA to the ends of chromosomes after each time the cell divides. Telomerase activity is very low or undetectable in normal cells although telomerase shows its high level of activity in cells that divide rapidly like cells that line the lungs and gastrointestinal tract, cells in bone marrow, and cells of the developing foetus. Telomerase allows these cells to divide many times without becoming damaged or undergoing apoptosis. Telomerase is also abnormally active in cancer cells, which grow and divide without control or order.

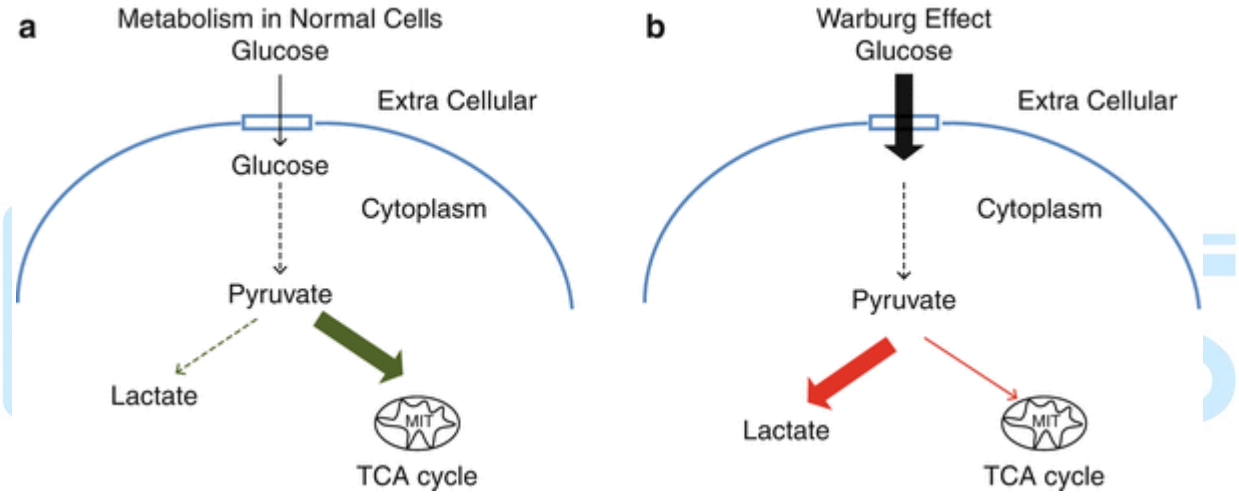
The telomerase enzyme consists of two major components that work together. The component produced from the *TERC* gene is known as hTR. The hTR component is an RNA molecule, a chemical cousin of DNA. It provides a template for creating the repeated sequence of DNA that telomerase adds to the ends of chromosomes. The other major component of telomerase, which is produced from a gene called ***TERT,*** is known as hTERT. The function of hTERT is to add the new DNA segment to chromosome ends.



4. **Sugar Metabolism:** Tumor cells shows rapid glucose uptake and faster glycolysis process about 10 times more rapid than a normal cells do. Most tumor cells shows hypoxic type of metabolism (that means cells grow with limited amount of oxygen supply) as they initially lack the capillary network to supply sufficient oxygen. At initial stage cancerous cells located more than 100 to 200 µm from the adjacent

capillaries, so they should depend upon glycolysis alone without further oxidation of pyruvate to yield more ATP. That means tumor cells initially yield 2 ATP instead of about 30 molecules of ATP as like normal cells using 1 molecule of glucose.

However in normal oxygen supply condition genetic mutation in tumor cells lead them to adopt glycolytic pathway to obtain ATP instead of aerobic oxidative phosphorylation. And so like muscle cells in hypoxic condition and developing embryo, tumor cells adopt glycolysis and lactate formation to meet their energy requirement. This metabolic adaptation of tumor cells is known as **Warburg Effect.** Tumor cells rapidly consume glucose in glycolysis mechanism to maintain its high rate of cell proliferation whatever the condition of the mitochondria may be they escape oxidative phosphorylation. To meet glucose supply glucose transporter mechanism highly active in tumor cells like $GLUT_3$.



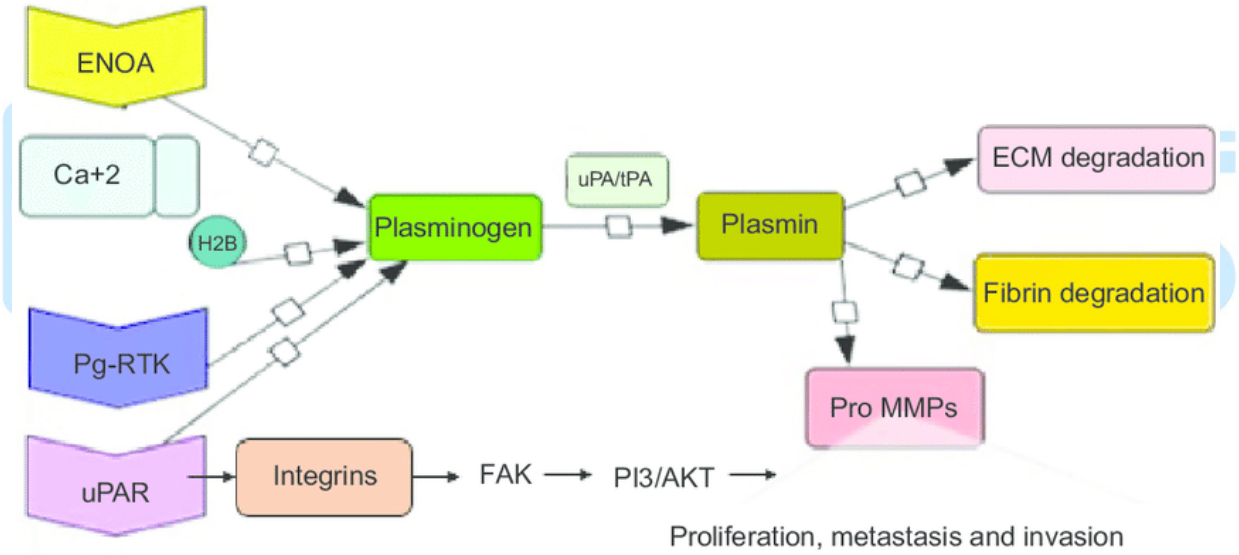
Escape Immune System of the Body:

Cancer cells are different form normal cells that means these cells may be treated as foreign particles by the immune system of the body. Two types of tumor antigens have been identified on tumor cells **tumor-specific transplantation antigens (TSTAs) and tumor-associated transplantation antigens (TATAs).** These tumor specific antigens are found in tumor cells and not occur on normal cells. Normally MHC-I molecule detects presence of antigens in the body. Mutation in the tumor cells prevent MHC-I molecule from detecting tumor specific antigens and thus tumor cells escape from the body's immune system. Malignant transformation of cells often is associated with reduction or even complete loss of MHC-I molecule. A various type of cancer shows to express decrease level of MHC-I molecule. And this lead to huge growth of tumor cells.

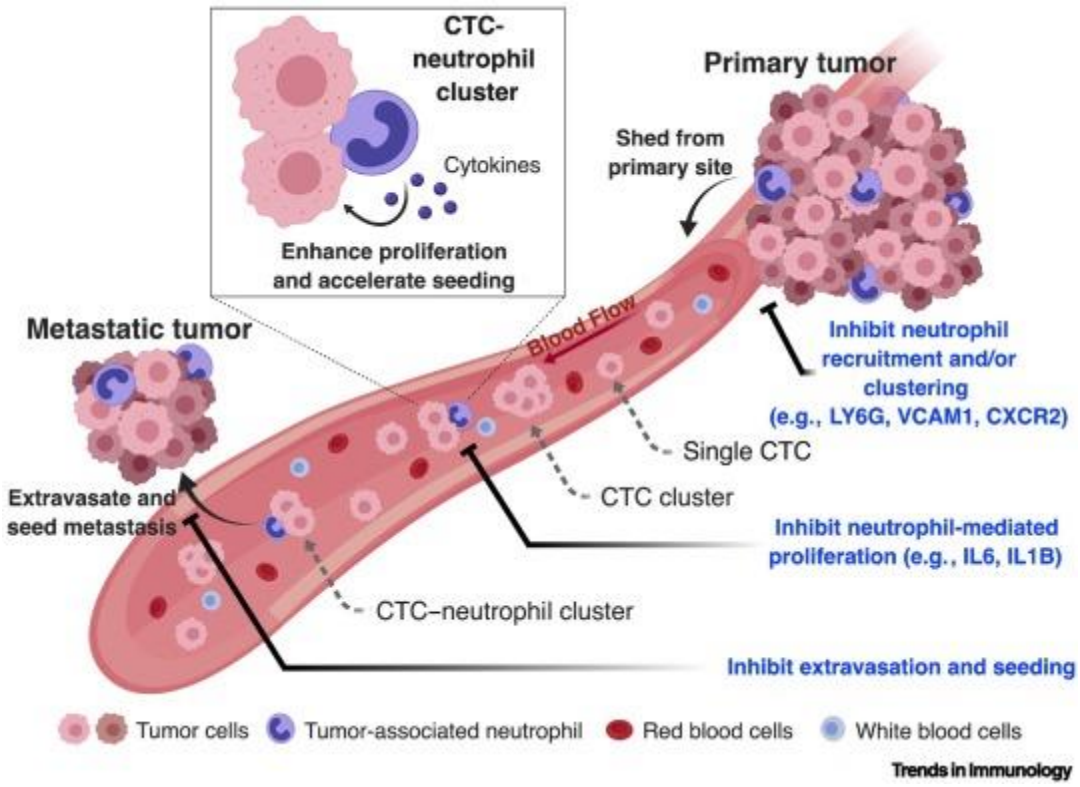
Invasiveness and Metastasis:

Invasiveness and metastasis are very crucial characteristics for malignant tumor cancer. Both invasiveness and metastasis are the means of spreading of tumor cells.

Invasiveness induce tumor on neighbouring cells whereas metastasis spread tumor on the distal region from the site of their origin by changing genetic characters of the cells which result in onset of cancer throughout the body. In case of benign tumor the cell adhesion molecule hold tumor cells within the tissue prevent them from being spread and localize them where they originated. Normal cells restricted their spreading by cell-cell adhesion molecules and basement membrane like physical barrier. Cancer cells have complex relation with the extracellular matrix and basement membrane. They penetrate the extracellular matrix using a special cell protrusion named **'invadopodium' formed by the localized assembly of an actin cytoskeleton.** Many tumor cells secret plasminogen activator protein which convert serum protein plasminogen to a active protease enzyme **plasmin.** High level of active plasmin protein along with other protease digest the basement membrane and make the tumor cells metastasized.



In addition, changes in cell surface protein and cytoskeleton protein found during the tumor cells formation and metastasis. Changes in the gene expression like Rho and GTPases that regulate the actin cytoskeleton. Tumor cells are found to have an over express of **RhoC gene** which encode a protein that promotes actin-myosin contraction in the cells and this increase actin myosin contraction or RhoC gene activity promote metastasis.



Angiogenesis:

Another most important characteristics of tumor cells that they shows high level of angiogenesis that means formation of blood vessels in the surrounding areas of the tumor cells to meet the high oxygen and nutrient requirement of the proliferating cells. Most tumor induce the formation of blood vessels that invade the tumor and nourish them. This mechanism of formation of new blood vessels is called angiogenesis.

Tumor cells produce several growth factors that stimulate angiogenesis. Basic fibroblast growth factors (bFGF), vascular endothelial growth factors (VEGF), transforming growth factor alpha (TGFα) are well known angiogenesis factors secreted by most of the tumor cells. Human express about five VEGF gene. VEGF expression can be induced by hypoxia, starvation of cells for oxygen that occurs when pO_2< 7mmHg. VEGF receptors are of Tyrosine Kinase family that regulate endothelial cells survival and their growth, cell migration and permeability of the vessel wall. The hypoxia condition signal is mediated by HIF-1 or **hypoxia-inducible factor-1.** These HIF-1 induce VEGF gene and several others gene which can affect the probability of the tumor growth. HIF-1 factor also help tumor cells to adapt to low oxygen by turning to glycolysis instead of oxidative phosphorylation for ATP generation. There are several natural protein that inhibit angiogenesis like **angiogenin and endostatin**

CELL CYCLE REGULATION AND CANCER:

Normal growth and development depends on a finely tuned highly regulated balance between growth between growth promoting and growth inhibiting factors. Genetic mutation that alter this balance may lead to onset of cancer.

TGFβ: Transforming growth factor β inhibit proliferation of many cells like epithelial cells and immune system cells. TGFβ bind to its receptor and induce activation of cytosolic **Smad transcription factor** and the Smad promote expression of the gene **p15,** an inhibitor of CDK4, which causes cells to arrest in G_1 phase. **TGFβ** also express genes that encode for extracellular matrix proteins and plasminogen activator inhibitor1 (**PAI-1)** which reduce the plasmin catalysed degradation of the matrix. Loss of functional activity due to mutation either in TGFβ receptor or in Smad promote cell proliferation and probably contribute to the invasiveness and metastasis. In human pancreatic cancers deletion of the of the Smad4 gene shown. In case of retinoblastoma and colon cancer cells lack of functional TGFβ receptors and therefore they became unresponsive to TGFβ induced growth inhibition.

Unregulated Passage from G_1 to S phase:

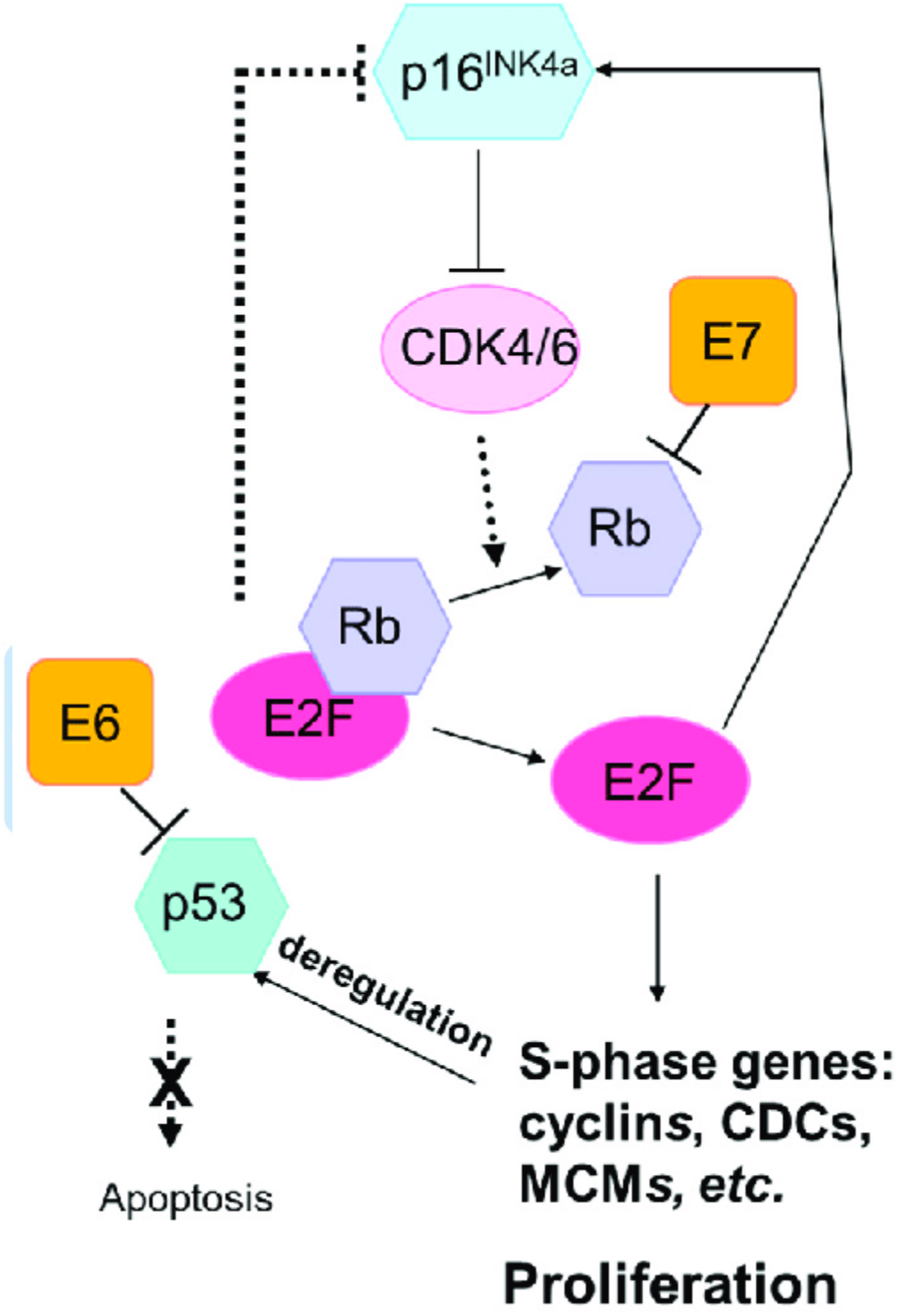
Once a cell progresses past a certain point in late G1, called the restriction point, it becomes irreversibly committed to entering the S phase and replicating its DNA. D-type cyclins, cyclin-dependent kinases (CDKs), and the Rb protein are all elements of the control system that regulate passage through the restriction point. The expression of D-type cyclin genes is induced by many extracellular growth factors, or mitogens. These cyclins assemble with their partners CDK4 and CDK6 to generate catalytically active cyclin-CDK complexes, whose kinase activity promotes progression past the restriction point. Mitogen withdrawal prior to passage through the restriction point leads to accumulation of p16. Like p15 mentioned above, p16 binds specifically to CDK4 and CDK6, thereby inhibiting their kinase activity and causing G1 arrest. Under normal circumstances, phosphorylation of Rb protein is initiated midway through G1 by active cyclin D-CDK4 and cyclin D-CDK6 complexes. Rb phosphorylation is completed by other cyclin-CDK complexes in late G1, allowing activation of E2F transcription factors, which stimulate transcription of genes encoding proteins required for DNA synthesis. The complete phosphorylation of Rb irreversibly commits the cell to DNA synthesis. Most tumors contain an oncogenic mutation that causes overproduction or loss of one of the components of this pathway such that the cells are propelled into the S phase in the absence of the proper extracellular growth signals.

High level of cyclin D1 found in many cancer. Amplification of cyclin D1 gene and overproduction of cyclin D1 protein id found in case of breast cancer. Cyclin D1 protein makes a cells to escape the G_1-S check point.

The proteins which function as cyclin-CDK inhibitors shows an important role in regulating the cell cycle. In particular, loss-of-functional mutations that prevent p16 from inhibiting cyclin D-CDK4/6 kinase activity are common in several human cancers. Loss of p16

mimics the overproduction of cyclin D1 protein that leads to Rb hyper phosphorylation and induce the release of active E2F transcription factor. So p16 in normal cells acts as a tumor suppressor. Although the p16 tumor-suppressor gene get deleted in some human cancers, in others the p16 sequence is normal. In these latter cancers (e.g., lung cancer), the p16 gene get inactivated by hyper methylation of its promoter region, that ultimately prevent transcription. The exact cause of this change in the methylation of p16 is not known, but it prevents production of this important cell-cycle control protein.

Mutations in both RB alleles lead to childhood retinoblastoma, a relatively rare type of cancer. However the loss of RB gene function also found in more common cancers that arise late stage of life (e.g., carcinomas of lung, breast, and bladder). These tissues, unlike retinal tissue, most likely produce other proteins whose function is redundant with that of Rb, and thus loss of Rb is not so critical. Several proteins are known that are related in structure and probably function to Rb. In addition to inactivating mutations, Rb function can be eliminated by the binding of an inhibitory protein, designated E7 that is encoded by human papillomavirus (HPV), another nasty viral trick to create virus-producing tissue.


p16INK4a
CDK4/6
E7
Rb
Rb
E6
E2F
E2F
p53
deregulation
Apoptosis
S-phase genes:
cyclins, CDCs,
MCMs, etc.
Proliferation

P53, ATM, ATR, Mdm2:

Functional p53 arrest cells in G_1 phase when these cells are expose to DNA damaging irradiation. Whereas cells that lack functional p53 cannot prevent cells to commit DNA replication in S phase with damaged DNA. DNA damage by γ-radiation or by heat or low oxygen level leads to activation of ATM or ATR, serine kinase that phosphorylate and thereby stabilize p53 leading to arrest of these DNA damaged cells in G_1 stage. About 50% cancers shows low level of p53 gene or mutations of p53 gene.

Under stressful condition the ATM kinase activity phosphorylate and activate a protein kinase Chk2 that phosphorylate the protein phosphatase Cdc25A and mark the stressful cells for ubiquitin mediated destruction. Mutations in ATM or Chk2 genes will result in decrease level of Cdc25A that will ultimately promote stressed cells to S phase for DNA replication.

The activity of p53 gene normally kept low by a protein called Mdm2. Mdm2 functions in an auto-regulatory feedback loop with by p53, perhaps normally preventing excess p53 function. The Mdm2 gene is amplified in many sarcomas and other human tumor cells. The high level of Mdm2 reduce the p53 concentration enough to abolish the p53 induced G_1 arrest in response to irradiation. The p53 activity is also inhibited by human papilloma virus.