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Molecular and Cellular Biology

Document intended for Master 1 Applied Biochemistry students

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Introduction

Introduction

The handout for this course is devoted to studying the stages of the cell cycle and the checkpoints of each stage. The cell cycle is the series of events in which cellular components are doubled, and then accurately segregated into daughter cells. In eukaryotes, DNA replication is confined to a discrete Synthesis or S-phase, and chromosome segregation occurs at Mitosis or M-phase. Two Gap phases separate S phase and mitosis, known as G1 and G2. The central machines that drive cell cycle progression are the cyclin-dependent kinases (CDKs).

Cell cycle progression is restrained by multiple checkpoint mechanisms that block transitions between cell cycle phases when cells encounter stressful conditions. For example, DNA damage activates checkpoints that delay cell cycle progression and trigger DNA repair. Under severe stress, DNA damage checkpoints may trigger permanent cellular outcomes such as apoptosis or senescence. Failure to fully activate DNA damage checkpoints can lead to genome instability, as the unrepaired DNA damage can be passed on to the next generation of cells. On the other hand, because cells routinely experience low levels of endogenous DNA damage, timely checkpoint recovery after DNA damage repair is necessary for continued cell proliferation. Therefore, the balance between cell cycle arrest and recovery must be regulated to continue proliferation in the face of constant exposure to endogenous and exogenous DNA damage sources.

Being the structural and functional unit of a living organism, cell has got a very important place in understanding the entire functioning of a living system. Therefore, it is required to have a thorough understanding of the structure and functions of a cell organelles. The second part of this document develops the structure and functions of the organelles constituting the eukaryotic cell and the mechanism of protein transport between these different cellular organelles (the biosynthesis/secretion and endocytosis pathways).

This handout is a very important educational support, intended for students who have the molecular and cellular biology module in their curriculum.

For educational purposes, the courses are simplified and structured in two chapters:

- The first concerns the different stages of the cell cycle and their regulation;
- The second is devoted to studying the structure and the different functions of the cellular organelles of eukaryotic cells, and the molecular mechanism of protein transport in the biosynthesis/secretion pathways.

Chapter I:
Cell cycle and its regulation

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1. Introduction

The cell cycle is the series of events in which cellular components are doubled, and then accurately segregated into daughter cells. In eukaryotes, DNA replication is confined to a discrete Synthesis or S-phase, and chromosome segregation occurs at Mitosis or M-phase. Two Gap phases separate S phase and mitosis, known as G1 and G2. These are not periods of inactivity, but rather periods where cells obtain mass, integrate growth signals, organize a replicated genome, and prepare for chromosome segregation. The central machines that drive cell cycle progression are the cyclin-dependent kinases (CDKs). These are serine/threonine protein kinases that phosphorylate key substrates to promote DNA synthesis and mitotic progression. The catalytic subunits are in molar excess, but lack activity until bound by their cognate cyclin subunits, which are tightly regulated at both the levels of synthesis and ubiquitin-dependent proteolysis. Cyclin-binding allows inactive CDKs to adopt an active configuration akin to monomeric and active kinases. Layered on top of this regulation, CDK activity can also be negatively regulated by the binding of small inhibitory proteins, the CKIs, or by inhibitory tyrosine phosphorylation which blocks phosphate transfer to substrates.

Cell cycle progression is restrained by multiple checkpoint mechanisms that block transitions between cell cycle phases when cells encounter stressful conditions. For example, DNA damage activates checkpoints that delay cell cycle progression and trigger DNA repair. Under severe stress, DNA damage checkpoints may trigger permanent cellular outcomes such as apoptosis or senescence. Failure to fully activate DNA damage checkpoints can lead to genome instability, as the unrepaired DNA damage can be passed on to the next generation of cells. On the other hand, because cells routinely experience low levels of endogenous DNA damage, timely checkpoint recovery after DNA damage repair is necessary for continued cell proliferation. Therefore, the balance between cell cycle arrest and recovery must be regulated to continue proliferation in the face of constant exposure to endogenous and exogenous DNA damage sources.

2. Cell cycle

2.1. Definition

The cell cycle is an ordered series of events involving cell growth and cell division that produces two new daughter cells from one parent cell. Cells on the path to cell division proceed through a series of precisely timed and carefully regulated stages of growth, DNA replication, and nuclear and cytoplasmic division that ultimately produces two identical cells.

The frequency of cell division varies depending on cell type and developmental stage. For example, during embryogenesis, cells divide rapidly as the embryo grows in size. Conversely, in an adult human body, almost all cells are terminally differentiated and are not dividing (example: neurons) or divide very infrequently (example: liver cells that divide about once per year). These non-dividing cells are termed quiescent cells, they are in the G_o stage.

2.2. Phases of cell cycle

The cell cycle is essentially divided into two major phases, interphase, during which the chromosomes are replicated, and mitotic phase, during which the chromosomes are distributed between the two daughter cells (Figure1).

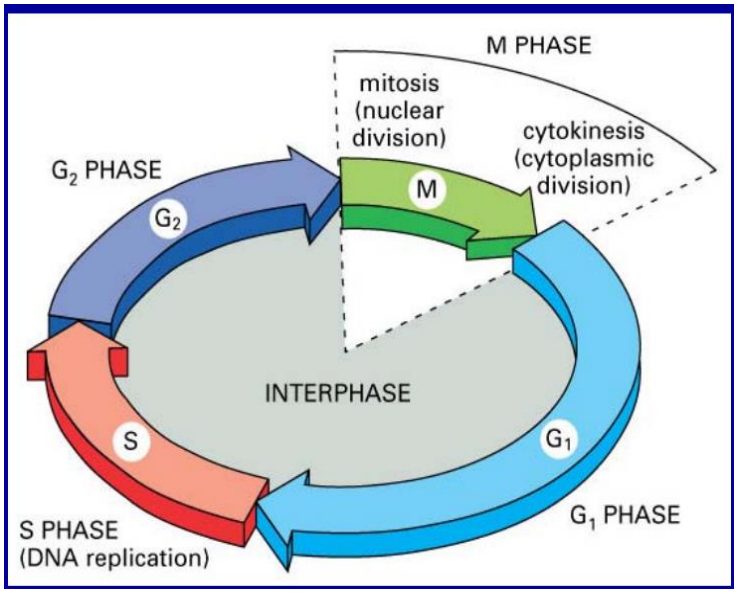

Figure 1: Phases of cell cycle.

2.2.1. M phase

Mitosis is the complex process that distributes the duplicated chromosomes equally into a pair of daughter nuclei. The pairs of sister chromatids are attached in early mitosis to the mitotic spindle, a bipolar array of protein polymers called microtubules. By the midpoint of mitosis (metaphase), sister chromatids in each pair are attached to microtubules coming from opposite poles of the spindle. At the next stage (anaphase), sister-chromatid cohesion is destroyed, resulting in sister-chromatid separation. The microtubules of the spindle pull the separated sisters to opposite ends of the cell (sister-chromatid segregation) and the two sets of chromosomes are each packaged into new daughter nuclei.

Mitosis is further divided into prophase, prometaphase, metaphase, anaphase, and telophase (Figure 2).

- Prophase is characterized with chromatin/chromosome condensation, centrosome separation, and nuclear membrane breakdown. The migration of centrosome to two opposite poles is important for the later formation of the bipolar mitotic spindle apparatus. A recent detailed study shows that the interphase organization is rapidly lost in prophase by a condensin-dependent manner.
- Prometaphase starts from the nuclear envelope breakdown. The microtubules composed of spindle fibres move from the pole to the centre of the cell (cells equator). Kinetochores attach themselves to specialized microtubules called kinetochore fibres. Kinetochores are special protein structures that develop on a chromatid during the process of cell division. It helps in attaching the spindle fibre to the chromosome. The kinetochore fibres combine with spindle polar fibres. Finally, the chromosomes start migrating toward the centre of the cell.
- Metaphase starts when the duplicated chromosomes are aligned along the metaphase plate in the middle of the cell. During metaphase, the sister chromatids are pulled back and forth by the kinetochore microtubules until they align along the equatorial plane. Once all the chromosomes are properly aligned and the kinetochores are correctly attached, the cohesion between sister

chromatids is dissolved, leading to the migration of the separated chromatids towards opposite sides of the cell by the pulling force of spindle microtubules.

- Anaphase onset is marked by the abrupt and synchronous separation of the sister chromatids, which is due to the sudden degradation of the cohesin complexes between the sister chromatids. During anaphase, the two sister chromatids, now daughter chromosomes, move to opposite spindle poles as their respective k-fibres shorten. This process is also referred to as anaphase A, to distinguish it from anaphase B, in which the spindle elongates, thus moving the two groups of segregating chromosomes further apart. In most organisms, there is temporal overlap between anaphase A and B.

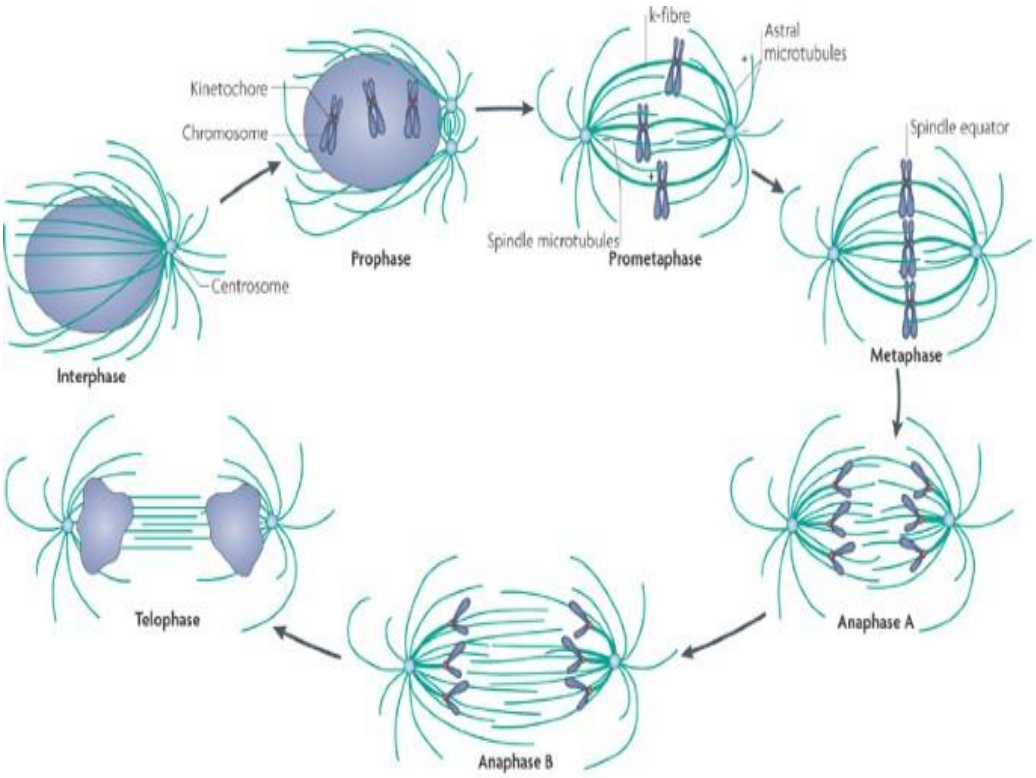

Figure 2: Different stages of mitosis according to chromosome movements.

- Telophase starts at the onset of the chromosome recondensation and the nuclear envelope reformation. During telophase the duplicated chromosomes in the nucleus of a parent cell separate into two identical daughter cells. A nuclear membrane forms around each set of chromosomes to divide the nuclear DNA from the cytoplasm. Simultaneously, the chromosome decondensation begins.

Cytokinesis normally starts during the later stages of mitosis (i.e. late anaphase/telophase), when a cleavage furrow containing an actin–myosin contractile ring forms and pinches the cell membrane of the dividing cell in a region corresponding to the spindle equator. This process divides the cytoplasm into two, thus completing cell division.

2.2.2. Interphase

Cells spend the majority of their time in interphase, which consists of three stages called Gap 1 (G1), Synthesis (or S), and Gap 2 (G2).

- **G1 phase:** The first gap phase (G1) corresponds to the interval between mitosis and initiation of DNA replication. In this phase, the cell is growing and replicating cytoplasmic organelles. It is also preparing for replication of its DNA by synthesizing the enzymes necessary to make copies of its DNA.
- **S phase:** In Synthesis (S) phase, the DNA is replicated and chromosomes are duplicated. This process begins at specific DNA sites called replication origins, which are scattered in large numbers along the chromosomes. At these sites, proteins open the DNA double helix, exposing it to the enzymes that carry out DNA synthesis, which move outward in both directions from the origins to copy the two DNA strands. Chromosome duplication also requires increased synthesis of the proteins, such as histones, that package the DNA into chromosomes. Additional proteins are deposited along the duplicated chromosomes during S phase, resulting in a tight linkage, or cohesion, between them. The duplicated chromosomes are called sister chromatids.
- **G2 phase:** During the Gap 2 phase (G 2), cell grows more, makes proteins and organelles, and begins to reorganize its contents in preparation for mitosis. During this phase, the replicated DNA undergoes another checkpoint. Here the DNA is checked for damage, which sometimes occurs in the synthesis phase of the cell cycle. After the cell passes this checkpoint, the DNA undergoes nuclear packaging. DNA is wound around proteins, creating chromatin fibers. The cell is now ready for mitotic division.

2.2.3. Duration of Cell Cycle

A typical eukaryotic cell cycle divide once in approximately every 24 hours. However, this duration of cell cycle can vary from organism to organism and also from cell type to cell type. The cell cycle is divided into mitosis and interphase. In cell cycle 95% is spent for interphase whereas the mitosis and cytokinesis last only for an hour (Table I).

For mammalian cells in culture the duration of S phase is 6-8 hours, M phase lasts for less than an hour. G2 is generally shorter than G1 and is more uniform in duration and usually lasts for 4-6 hours. The length of G1 is quite variable, depending on the cell type. A typical G1 lasts for 8-10 hours, some cells spend only minutes or hours in G1 where as other spend weeks, month or years. During G1, a major decision is made as to whether and when the cell would divide again. Cells that are arrested in G1, for long periods are said to be is a Go state.

Those tissues that normally do not divide (such as nerve cells or skeletal muscle) or that divide rarely such as circulating lymphocytes, contain the amount of DNA present in G1 period. Cultured cells that slop multiplying because of density dependent inhibition of growth (or contact inhibition) also stop at G1.

For a typical proliferating human cell, the duration of G1 phase is approximately 11 h, S phase duration last 8 h, G2 phase last 4 h, and the duration of M phase is approximately 1 h. Cells that are arrested in G1, for long periods are said to be is a Go state (Table1).

Table I: Duration of cell cycle.

Phase	Time duration (in hrs)
G_1	11
S	8
G_2	4
M	1

2.2.4. Cell Cycle Checkpoints

Because errors encoded in the genome may result in defective clones, close monitoring of the cell cycle for abnormal programming is mandatory. The best understood and probably the most important regulatory site is the checkpoint referred to as the "restriction point" (R) in the latter third of G1. An error occurring later in the cell cycle, in S or at G2/M, is recognized by other checkpoints: depending upon the degree of damage, either the defect will be repaired or mitosis will be aborted.

Checkpoint is an important control point present in the cell cycle where stop and start signals can regulate the cell cycle (Figure 3).

- **G1-restriction checkpoint:** G1-restriction checkpoint decides whether the cell will enter into the G0 phase or S phase. G1-restriction point checks if the cell is fully mature, with ample space and adequate supply of nutrients, then the growth hormone will induce S-phase. Otherwise, if the cell maturation is incomplete with inadequate space, nutrients, and growth factors, then the cell is destined to enter G0.
- **S-checkpoint:** S-checkpoint ensures the completion of DNA duplication (replication). At S-checkpoint by default, DNA is completely replicated with rising levels of DNA polymerase with its substrate (dNTPs) and cyclins. There is one important controlling mechanism that comes into play, termed as "replication licensing," otherwise known as "The Fidelity Check of Cell Cycle." It takes place by means of phosphoric degradation of origin binding protein, which is essential for the replication. It is the intrinsic property of an individual chromosome, which ensures single replication per each cell cycle.
- **G2-DNA damage checkpoin**t: G2-DNA damage checkpoint checks preparedness of the cell for mitosis. At G2 checkpoint, there occur peak levels of cyclin with increased activation of mitosis-promoting factor (MPFkinase), which results in nuclear envelope disintegration. It also ensures that DNA replication in the S-phase has been completed

successfully. It monitors the levels of growth factors and other related proteins as well.

- **M (metaphase)-spindle assembly checkpoint:** M-spindle assembly checkpoint ensures that all sister chromatids are ready for separation. The M-spindle assembly/integrity checkpoint operates at the end of M-phase in order to sense proper spindle integrity, precise chromosomal orientation in metaphase plate, and appropriate binding of spindle fibers to chromosomes. If any of these three found defective, then the integrity checkpoint stops the cell cycle progression, thereby preventing inappropriate "chromosomal sorting (Figure 3).

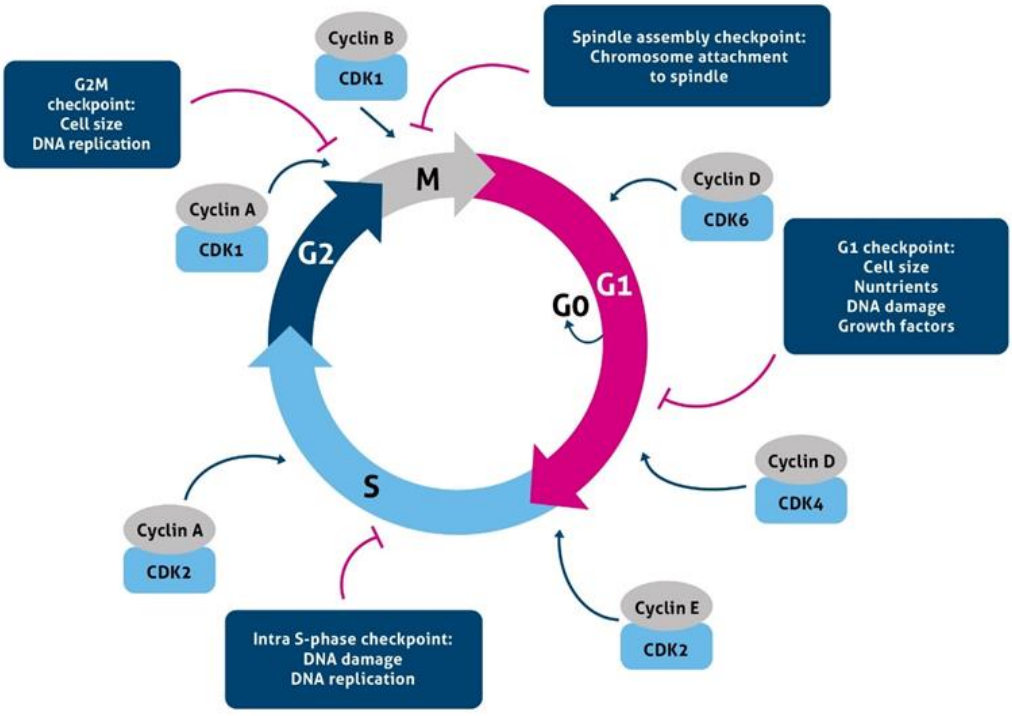

Figure 3: The cell cycle and its checkpoints.

3. Cell cycle regulation

3.1. Regulation of cell cycle by cyclin-dependent kinases (CDKs)

3.1.1. Definition and mode of action of cyclin-dependent kinases (Cdk)

The central machines that drive cell cycle progression are the cyclin-dependent kinases (Cdks). These are group of protein kinases (serine/threonine kinases), activated

via formation of a complex with cyclin molecules, involved in cell cycle regulation. Cyclin is the regulatory unit and CDK is its catalytic partner (Figure 4).

- **Cyclin:** Cyclin proteins are named so, due to their cyclic nature of production and degradation. They mainly trigger kinase activity. About 8 cyclins have been identified. Cyclin D, E, A, and B come to play serially during each cell cycle.

  The levels of the different cyclins vary considerably across the cell cycle, as shown in the diagram at right. A typical cyclin is present at low levels for most of the cycle, but increases strongly at the stage where it's needed. M cyclin, for example, peaks dramatically at the transition from G2 to M phase. G1 cyclins are unusual in that they are needed for much of the cell cycle.
- **Kinase**: These are the enzymes affecting the molecular activity of those proteins, which help a cell to pass through various stages of the cell cycle.

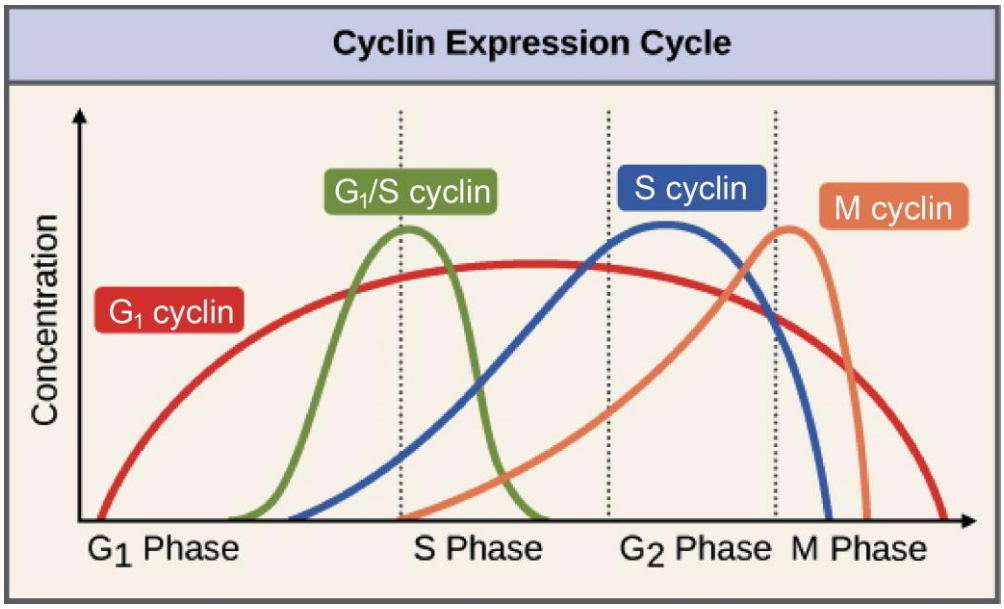

Figure 4 : Cyclin expression during cell cycle.

Cyclin/Cdk complexes phosphorylate specific protein substrates to move the cell through the cycle with activation of DNA synthesis (in late G1 and S), and formation of the structural components associated with mitosis (in late G2 and M). The periodicity of the cyclins, mediated by their synthesis and subsequent proteolytic degradation, ensure the well-delineated transitions between cell cycle stages.

Their activity consists of transferring the γ-phosphate group of ATP onto a serine or threonine, present in the target proteins, provided that these amino acids are

in a characteristic amino acid sequence (consensus sequence) specifically recognized by the kinase (example: Ser/Thr-Pro-X-Arg/Lys). This phosphorylation results in a change in conformation of the target proteins, which results in new properties for the latter (activation, inhibition, change of interaction partner, etc.)

3.1.2. Intervention of complexes cyclin/Cdks in the progression of the cell cycle

The cycle begins in G1 with increased expression of the D cyclins. The D cyclins associate with CDK4 and CDK6; formation of the cyclin/CDK complexes results in phosphorylation and activation of the CDKs. The activated CDKs then phosphorylate the retinoblastoma (RB) protein. The RB protein has a critical role in regulating G1 progression (Figure 5).

The RB family members are proteins that sequester the E2F transcription proteins. Unphosphorylated RB tightly binds E2F and inhibits transcription. Upon RB phosphorylation by CDK4/6, RB dissociates from E2F, allowing E2F to transcribe a number of responder genes (including cyclin E) required for passage through R. The phosphorylation of RB is associated with release of E2F and passage through R. RB is maintained in its phosphorylated state throughout the remainder of the cycle ; it may play a role in guiding the cell through S, G2, and M. RB is not dephosphorylated until mitosis is complete.

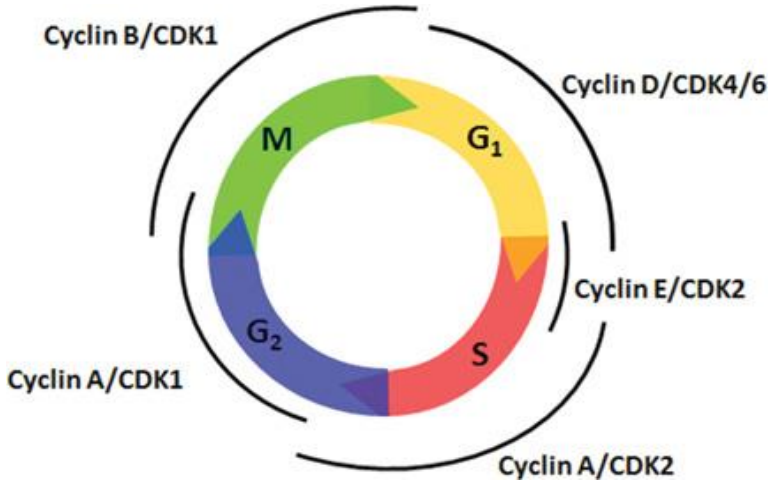

Figure 5: Intervention of complexes cyclin/Cdks in the progression of the cell cycle.

Different CDK-cyclin complexes drive the individual phases of the cell cycle. Chromosomal DNA is replicated during the synthesis (S) phase; replicated chromosomes are segregated to daughter nuclei in M phase (mitosis). Gap phases G1 and G2 of variable duration may separate M from S and S from M, respectively.

As the cell progresses through late G1, there is increased expression of cyclin E. The cyclin E/CDK2 complex is required for the transition from G1 into S. Increased expression of cyclin A occurs at the G1/S transition and persists through S phase. With the binding of cyclin A to CDK2, DNA synthesis proceeds. In the latter part of S, cyclin A associates with CDK1. Increased levels of cyclins A and B complexed with CDK1 propel the cell through mitosis.

Table 2: The Cdk/cyclin complexes formed during cell cycle regulation and their functions.

Cdk	Associated Cyclin	Cell-Cycle Stage
Cdk1	Cyclin A, B	G_2/M
Cdk2	Cyclin A, D, E; cyclin H	G_1/S; S; G_2/M
Cdk3	Ik3–1, Ik3–2	G_1
Cdk4	Cyclin D	G_1/S; S
Cdk5	Cyclin D	G_1/S
Cdk6	Cycin D	G_1/S; S
Cdk7	Cyclin H	G_1/S; transcriptional regulation
Cdk8	Cyclin C	G_1/S; G_2/M, transcriptional regulation
Cdk9	Cyclin T1, T2	Acts on differentiation, interaction with tat, the transcriptional regulator of the HIV virus
Cdk10	Interacts with ets-2[25]	G_2/M[27]
Cdk11	RanBPM, RNPS1[26] casein kinase[58], cyclin L	Promotes apoptosis
Cdk12	Cyclin L1 and L2	Regulates alternative splicing[29]
Cdk13	Cyclin L	Regulates alternative splicing[10]

3.2. Regulation of Cdk activity

There are several mechanisms for regulating the activity of CDKs, so that these CDKs can be activated or inactivated at just the right time.

3.2.1. Positive regulation of the cell cycle

The CDK activity is regulated by their association with the cyclins. Their name derives from the fact that their concentration varies cyclically during the cell cycle; their synthesis and degradation depends on the different stages of the mitotic cell division cycle. CDKs are additionally controlled by a series of kinases and phosphatases other than cyclins.

- **Cdk Activating Kinase (CAK)**

  The best example of such positive regulator is CDK activating kinase (CAK) which is known to phosphorylate threonine residues at the CDK active sites. CAK activity is constant throughout the cell cycle and phosphorylates the CDK as soon as a cyclin/CDK complex is formed.

  CDK7 together with cyclin H and the assembly factor MAT1 (ménage a trois) forms the CDK activating kinase (CAK) complex, responsible for the activating phosphorylation of CDK1, CDK2, CDK4 and CDK6.

  a. ***Cyclin-Dependent Kinase 1 (CDK1):*** Cyclin-dependent kinase 1 (CDK1) interacts with cyclin B1 to facilitate the transition from the G2 phase into mitosis. This enzyme is further controlled by checkpoint kinases, such as Wee1-like protein kinase (WEE1) and checkpoint kinase 1 (CHK1), which ensure that incompletely replicated or damaged DNA is not distributed to daughter cells. CDK1/cyclinB activity starts to increase in late G2, and continues through prometaphase until the spindle assembly checkpoint is satisfied and the cell enters the metaphase. This complex is activated through the CDC25-mediated dephosphorylation of inhibitory phosphorylation on Thr14 and Tyr15

  b. ***Cyclin-Dependent Kinase 2 (CDK2)****:* In dividing cells, cyclin-dependent kinase 2 (CDK2) is a major cell cycle component that controls the G1/S and S/G2 transitions. CDK2/CyclinE must phosphorylate Rb to induce S phase entry.

  c. ***Cyclin-Dependent Kinases 4 and 6 (CDK4/6):*** Direct inhibition of the cyclin D-CDK4/6 dimer activity prevents cell cycle progression from the G1 to the S phase of the cell cycle. This tightly controlled restriction point is

regulated by CDK4/6 complex. Activated CDK4/6 complexes are responsible for the phosphorylation of retinoblastoma gene product (Rb) by functionally inactivating it (Figure 6). Phosphorylation of Rb allows dissociation of the transcription factor E2F from the Rb/E2F complexes, thus facilitating the subsequent transcription of E2F target genes, such as those for the E-type cyclins (cyclins E1 and E2). By interacting with CDK2, cyclin E hyperphosphorylates RB, further increasing the activity of the E2F target genes, which are needed for initiation of DNA synthesis and entry into the S phase, thereby allowing the cell to proceed through the cell cycle and divide.

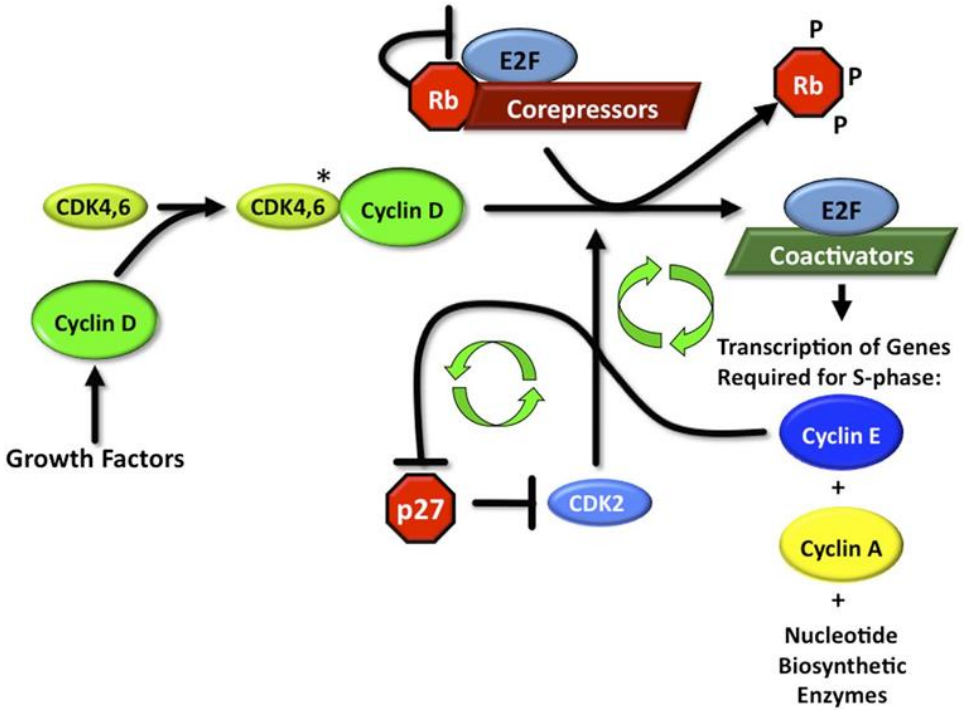

Figure 6: Retinoblastoma (Rb) binds to and inhibits the transcription factor E2F. *Extracellular growth factors upregulate D-type cyclins through transcriptional, translational, and post-translational mechanisms, resulting in activation of CDK4 and 6. CDK4/6 initiates Rb phosphorylation, causing partial activation of E2F-dependent transcription, which leads to induction of cyclins E and A and the activation of other genes required for DNA synthesis. Cyclin E/CDK2 complexes phosphorylate p27, triggering its ubiquitination and proteasomal degradation. CyclinE/CDK2 complexes also phosphorylate the pocket proteins Rb, p107, and p130 on additional sites, further promoting E2F-dependent transcription. The two embedded positive feedback loops ensure that once cells have traversed the restriction point, they are committed to a round of replication.*

- **Phosphatases CDC25**

Cell Division Cycle-25 (CDC25) plays an important role in transitions between cell cycle phases by dephosphorylating and activating CDKs. In higher-eukaryotic cells, there exist three Cdc25 family members, Cdc25A, Cdc25B, and Cdc25C. While Cdc25A plays a major role at the G_1-to-S phase transition, Cdc25 B and C are required for entry into mitosis. Cdc25B undergoes activation during S-phase and plays a role in activating the mitotic kinase Cdkl/cyclin B in the cytoplasm. Active Cdkl/cyclin B then phosphorylates and activates Cdc25C leading to a positive feedback mechanism and to entry into mitosis (Figure 7).

CDC25A is mainly localized in the nucleus, and controls G1/S progression by dephosphorylation-dependent inactivation of Cyclin E/CDK2 and Cyclin D/CDK4-6. CDC25B is located in the nucleus during interphase, but shuttles back and forth between the cytoplasm and nucleus during entire cell cycle. It translocate to the cytoplasm during the G2 phase and activates the cyclin B1/ CDK1 complex, and then re-enters the nucleus to initiate mitosis.

In late G2-phase of the cell cycle the cdc25C phosphatase dephosphorylates cdk1 of the cyclin B1/cdk1 complex at both threonine 14 and tyrosine 15 leading to the activation of the cyclin B1/cdk1 complex and to entry into mitosis. The activity of cdc25C is strictly regulated throughout the cell cycle. The phosphatase activity of cdc25C is low during interphase and enhanced during mitosis. Mitotic activation of cdc25C is achieved by phosphorylation in the amino terminus of cdc25C by cdk1 and by polo-like kinases. The 14-3-3 protein is a phosphoserine binding protein that negatively regulates cdc25C. During interphase cdc25C is phosphorylated at serine 216 and bound to the 14-3-3 protein whereas during mitosis cdc25C is phosphorylated at serine 214 which prevents phosphorylation at serine 216 and binding of the 14-3-3 protein. Binding of cdc25C to the 14-3-3 protein leads to a nuclear exclusion of cdc25C and thus to inactivation of cdc25C.

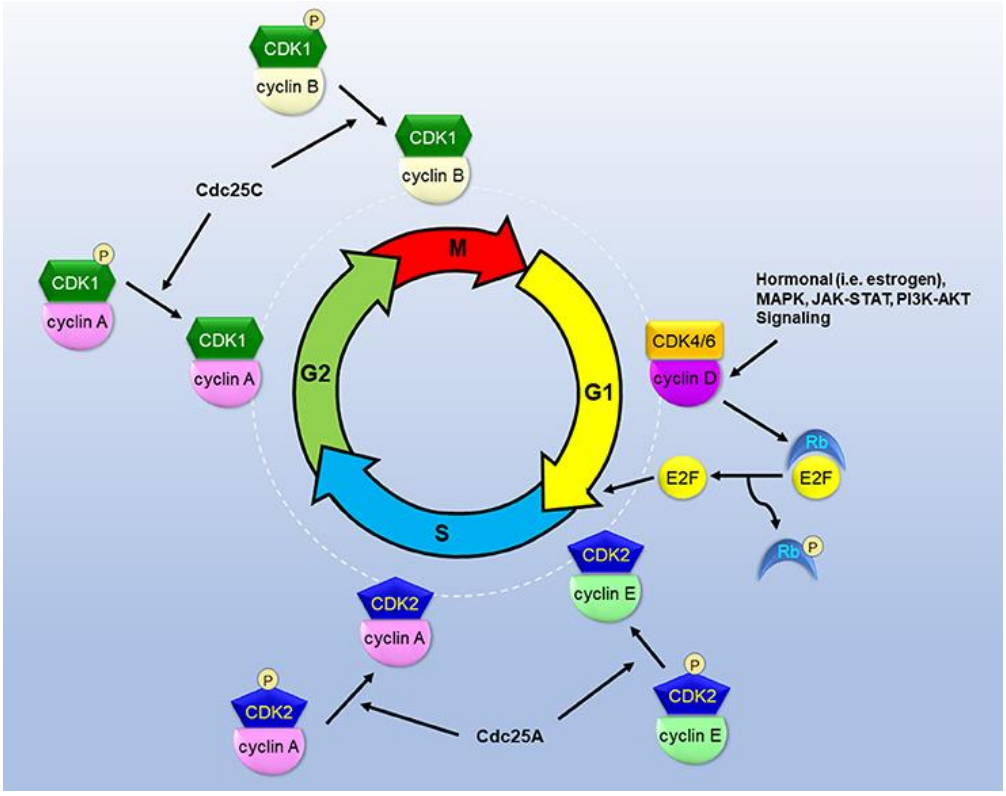

Figure 7: Positive regulation of the cell cycle by CDC 25. *CDKs bind to specialized cyclins to form active complexes that drive cell cycle phase progression and transition into next phases. Growth and mitogenic signals induce cyclin D and activate CDK4, thereby inactivating Rb and releasing E2F to instigate G1 phase progression. Cdc25 phosphatases dephosphorylate and activate CDKs to promote S/G2/M phase progression.*

3.2.2. Negative regulation of the cell cycle

- **Wee 1 family**

  The Wee1 family of kinases, comprised of Wee1 and Myt1, function to inhibit Cdk1/Cyclin B thus inducing interphase and preventing entry into mitosis. While both Wee1 and Myt1 can phosphorylate Tyr15 of Cdk1 in order to restrict its catalytic activity, Myt1 can also phosphorylate Thr14, which has been shown to negatively regulate Cdk1 as well. Wee1 is a predominantly nuclear protein that prevents active Cdk1/Cyclin B from accumulating in the nucleus during interphase. In contrast, Myt1 is membrane-bound and can both inactivate Cdk1 via phosphorylation and bind Cdk1, thus sequestering Cdk1/Cyclin B at the membrane. Both Wee1 and Myt1 are active during interphase and become inactivated at the G2/M transition (Figure 8).

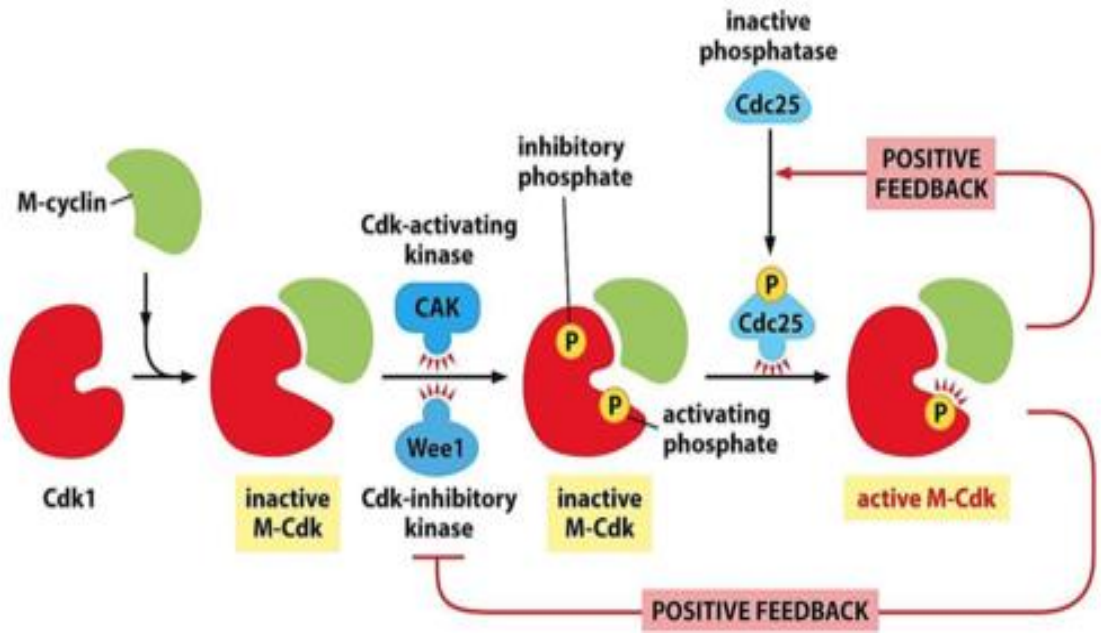

Figure 8: Activation of mitotic-cyclin/Cdk complex (MPF: maturation promoting factor). *As cyclin B is synthesized during S and G2 phases of the cell cycle, it associates with Cdk1. Active CDK-activating kinase (CAK) phosphorylates Cdk1 at threonine 161, stabilizing its association with cyclin B, but the complex is not immediately active because the Cdk1 subunit is phosphorylated by other kinases at inhibitory sites, threonine 14 and tyrosine 15. Final activation of MPF occurs when these sites are dephosphorylated by CDC25 phosphatase. The MPF activates its own activator (CDC25 phosphatase) and inhibits its own inhibitor (Wee 1), as part of a positive feedback mechanism.*

- **P16 and P 21 families**

CDK activity is regulated negatively by small inhibitory proteins. There are two distinct families of CKIs: the INK4 family (p16, p15, p18 and p19) and the Cip/Kip family (p2, p27and p57). The first family specifically inactivate CDK4 and CDK6, which prevents their combination with D-type cyclins. However the proteins of the second family form heterotrimeric complexes with cyclin D-, cyclin E-, and cyclin A-dependent kinase complexes (Figure 9 and 10).

Phosphorylation of cyclin-dependent kinases (CDKs) by the CDK-activating kinase is required for the activation of CDK enzymes. Members of two families of CDK inhibitors become physically associated with and inhibit the activity of CDKs in response to a variety of growth-modulating signals (Figure 10).

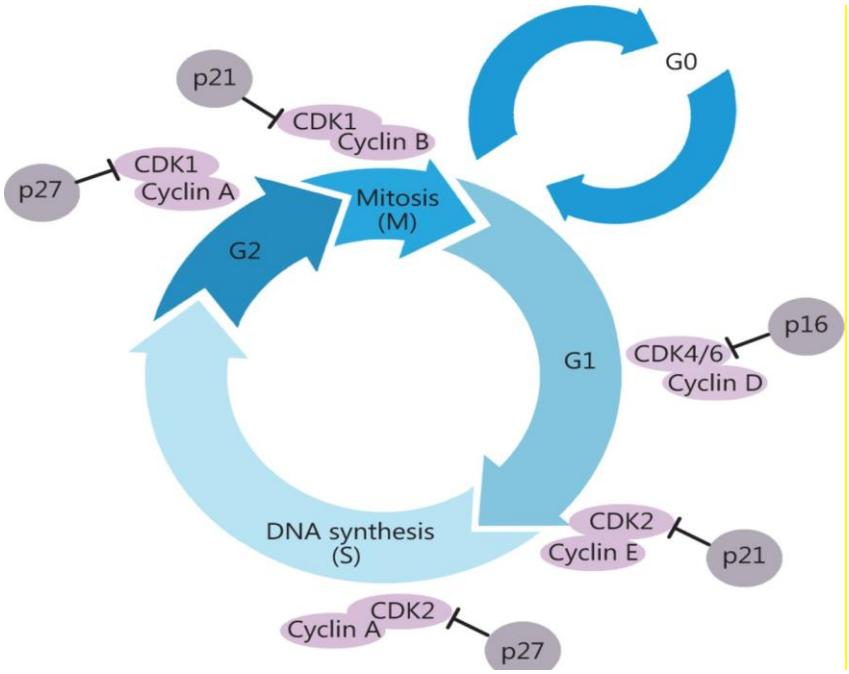

Figure 9: CKI regulation in the cell cycle. *Two major classes of CDK inhibitors have been identified. The p16 family (p15, p16, p18 and p19) binds to and inhibits the activities of CDK4 and CDK6. The p21 family (p21, p27, p28 and p57) can bind to broad range of CDK-cyclin complexes and inhibit their activities.*

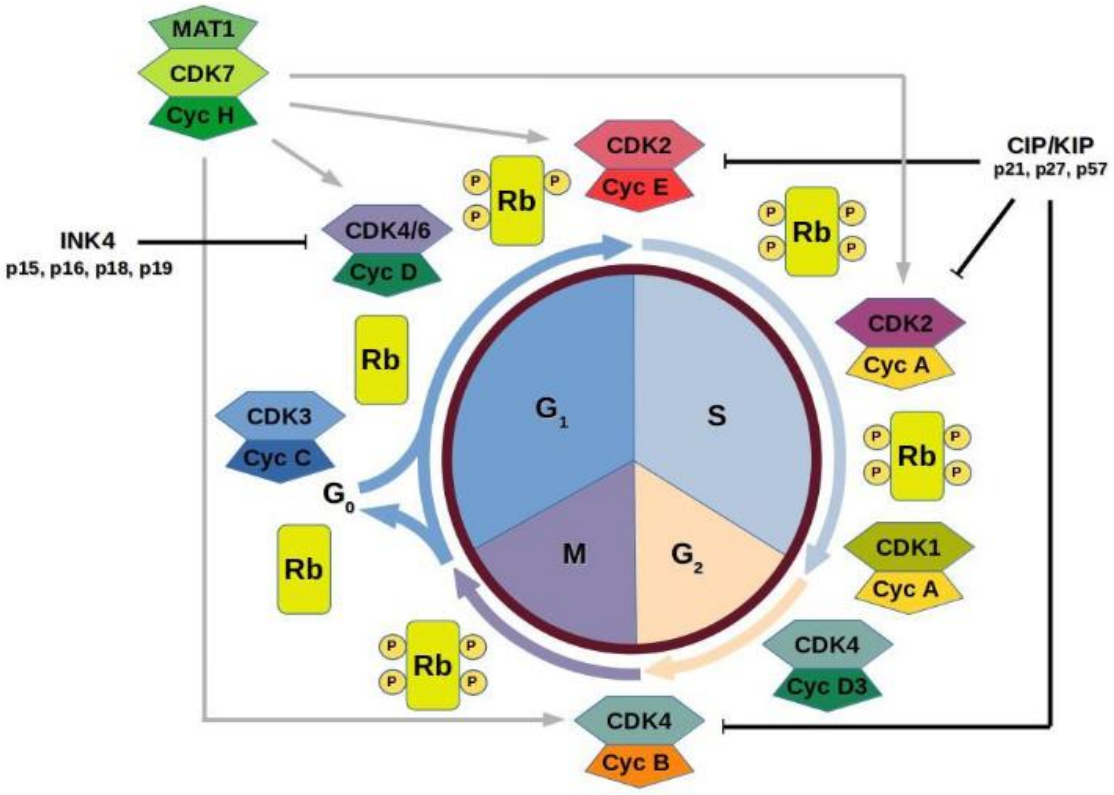

Figure 10: Positive and negative regulation of the cell cycle.

4. Entry into mitosis (G2/M transition)

4.1. Introduction

The G_2/M transition is a decisive point in a cell's life cycle. The point at which, after successfully completing a second growth phase (G2 phase) following the replication of its DNA (S phase), it begins mitosis (M phase), the phase during which it physically separates itself into two daughter cells

Maturation or M phase-promoting factor (MPF) is the universal inducer of M phase common to eukaryotic cells. MPF was originally defined as a transferable activity that can induce the G2/M phase transition in recipient cells. Today, however, MPF is assumed to describe an activity that exhibits its effect in donor cells, and furthermore, MPF is consistently equated with the kinase cyclin B/Cdk1.

4.2. Regulation of entry into mitosis

Entry into mitosis can be broken down into three stages:

- Formation and accumulation of inactive Cdk1/Cyclin B complexes (pre-MPF).
- 2. Activation of inactive Cdk1/Cyclin B complexes.
- 3. Action of Cdk1/Cyclin B complexes.

4.2.1. Formation and accumulation of inactive Cdk1/Cyclin B complexes (pre-MPF)

The transition from the second Gap phase (G_2) to mitosis is regulated by CDK1 (formerly Cdc2) in association primarily with cyclin B. Like other CDKs, CDK1 is relatively stable, and activation depends first on accumulation of cyclin B. Mitotic cyclins accumulate during S phase and associate with CDK1; however, this complex is maintained in an inactive form via two mechanisms. In the first, Wee/Myt1-dependent phosphorylation of Thr14/Tyr15 prevents ATP binding. The second mechanism relies on active transport of CDK1/cyclin complexes out of the nucleus. Onset of mitosis is triggered by dephosphorylation of CDK1 by a CDC25 isoform and increased nuclear transport/decreased nuclear exit of CDK1/cyclin complexes (Figure 11).

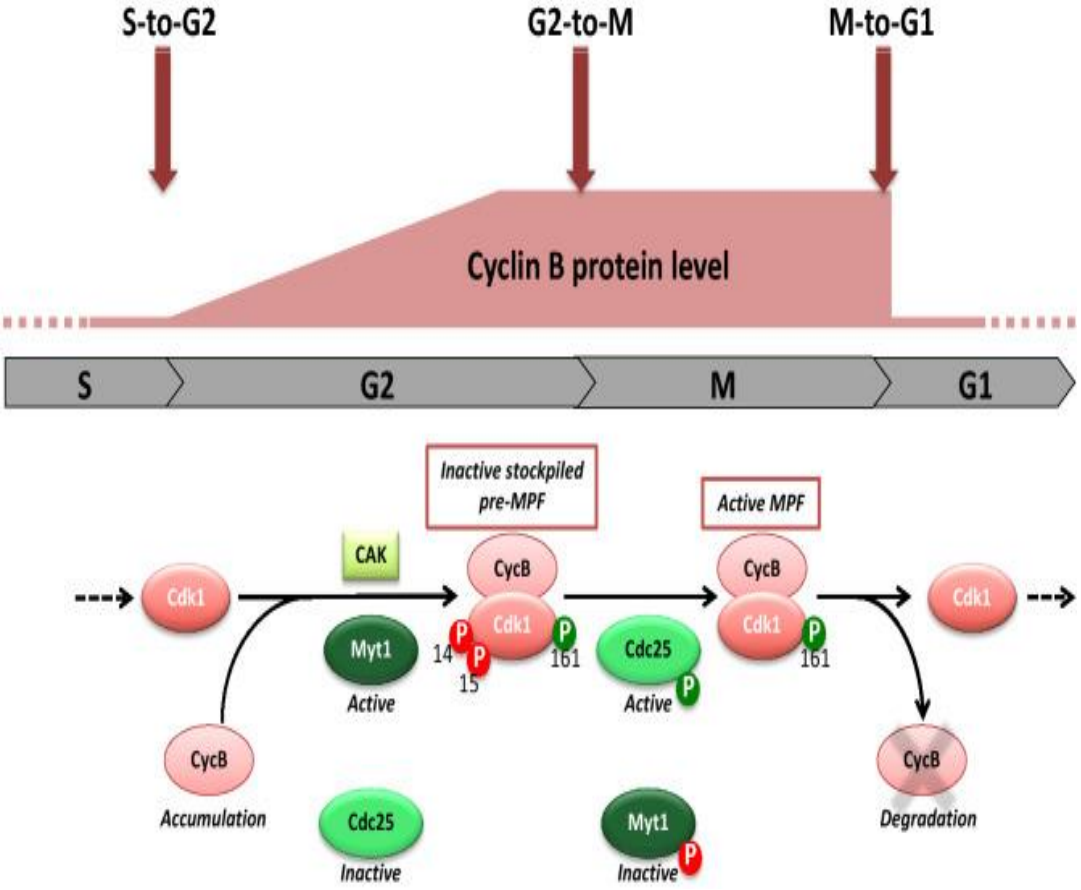

Figure 11: The three transitions of the mitotic cycle involving Cdk1/Cyclin B. *During the G2-phase, Cyclin B accumulates and binds to Cdk1 that is phosphorylated at Thr 161 by CAK and at Tyr15and Thr 14 by Wee1/Myt1. The complex is thus inactive. At the G2-to-M transition, Wee1/Myt1 are turned off while CDC25 is turned on, promoting Tyr15 and Thr 14 dephosphorylations and Cdk1 activation. During M-phase, Cdk1/Cyclin B phosphorylates many mitotic substrates, including Wee1/Myt1 and Cdc25. At the end of M-phase, MPF activates the Cyclin proteolytic degradation system, thus inducing its own inactivation.*

4.2.2. Activation of inactive Cdk1/Cyclin B complexes

Activation of the Cyclin B/Cdk1 stock is the result of a series of interdependent reactions (Figure 12):

When replication is completed, the Cdc25 phosphatase is no longer sequestered in the cytoplasm and passes into the nucleus, and at the end of G2 phase, the Cdc 25 phosphatase is activated, by phosphorylation by Polo kinase. Cdc 25 then has the action of removing the two inhibitory phosphates of Cyclin B/Cdk 1–at Thr14 and

Tyr15, converting Cdk1/Cyclin B into active MPF. This allows the activation of a small part of the stock of Cyclin B / Cdk1.

Cdk 1 can then phosphorylate Cdc 25 (on a site different from that of Polo kinase). This phosphorylation activates Cdc 25: Cyclin B / Cdk 1 therefore activates its own activator (self-activation process). At the same time, Cyclin B/Cdk 1phosphorylates Wee 1, inhibiting this protein: Cyclin B/Cdk1 therefore inhibits its own inhibitor.

This double positive feedback mechanism makes it possible to quickly and irreversibly obtain a large quantity of active Cyclin B/Cdk1 complexes (Figure 11 (b)).

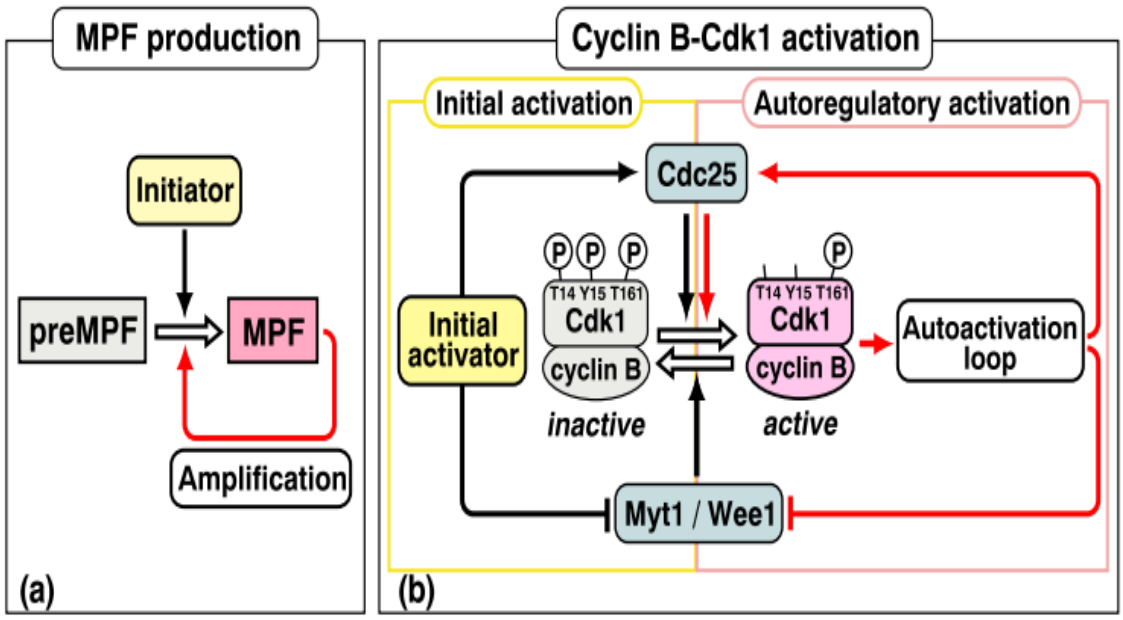

Figure 12: Initial activation and amplification of MPF.

(a) *Initiator activates a small amount of pre-MPF to MPF (initial activation), and thereafter, MPF itself activates large amounts of pre-MPF to MPF (amplification). (b) Initial activation and autoregulatory activation of cyclin B/Cdk1. At the initial onset of the G2/ M phase transition, a putative initial activator reverses the balance between Cdc25 and Myt1/Wee1 to trigger activation of a small amount of cyclin B-Cdk1 (initial activation), and thereafter, the active cyclin BCdk1 starts the autoactivation loop to induce activation of large amounts of cyclin B/Cdk1 (autoregulatory activation).*

4.2.3. Action of Cdk1/Cyclin B complexes

The MPF (maturation promoting factor) complexes add phosphate tags to several different proteins. It plays a role in chromatin condensation by phosphorylating condensins; a role in NEBD (nuclear envelope breakdown) by phosphorylating lamins, nuclear pore complexes, and inner nuclear membrane proteins; a role in fragmentation of the Golgi apparatus by phosphorylating Golgi matrix proteins; and a role in spindle formation by phosphorylating centrosome-associated proteins and MAPs (Figure 13).

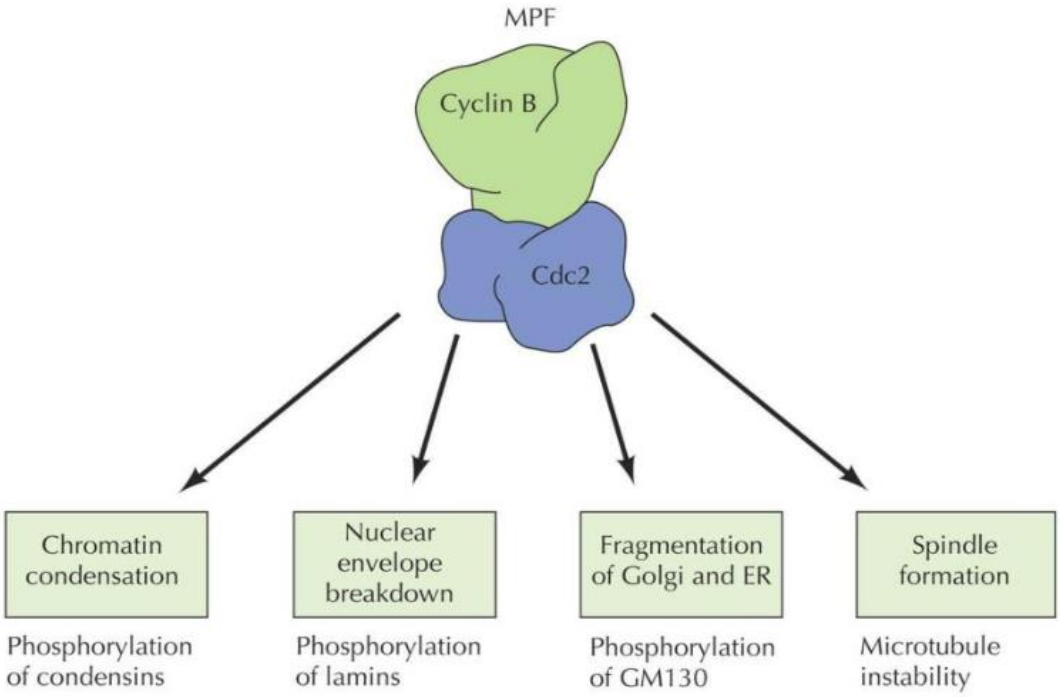

Figure 13 : Targets of MPF.

- **Nuclear envelope breakdown:** During mitosis, a single nucleus gives rise to two nuclei that are identical to the parent nucleus. Mitosis consists of a continuous sequence of events that must be carried out once and only once. Two such important events are the disassembly of the nuclear envelope (NE) during the first stages of mitosis, and its accurate reassembly during the last stages of mitosis. NE breakdown (NEBD) is initiated when maturation-promoting factor (MPF) enters the nucleus and starts phosphorylating nuclear

pore complexes (NPCs) and nuclear lamina proteins, followed by NPC and lamina breakdown.

Phosphorylation of lamins (intermediate filaments of the nucleus) by Cyclin B/Cdk 1 at the start of mitosis causes their depolymerization. However, these laminas were associated with the nuclear envelope, and made it possible to structure it. Consequently, their depolymerization has the effect of disorganizing the nuclear envelope which disperses into small vesicles. Certain lamins (B lamins) remain anchored in nuclear envelope vesicles because they are isoprenylated (lipid anchor) (Figure 14). Lamins have chromosome-binding sequences, which facilitate the reformation of the nuclear envelope around them at the end of mitosis. The opposite process takes place at telophase, at the end of mitosis, when the lamins will be dephosphorylated (Figure 15).

The destruction of the nuclear envelope is important because it allows the mitotic spindle to access the chromosomes.

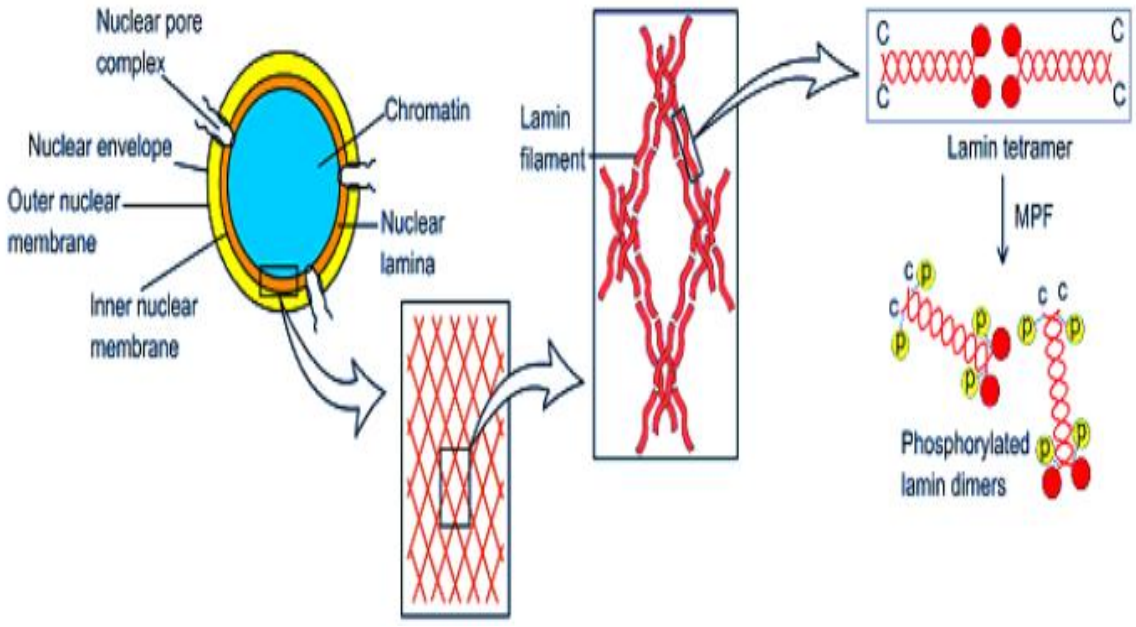

Figure 14: Phosphorylation of lamins (intermediate filaments of the nucleus) by Cyclin B/Cdk 1.

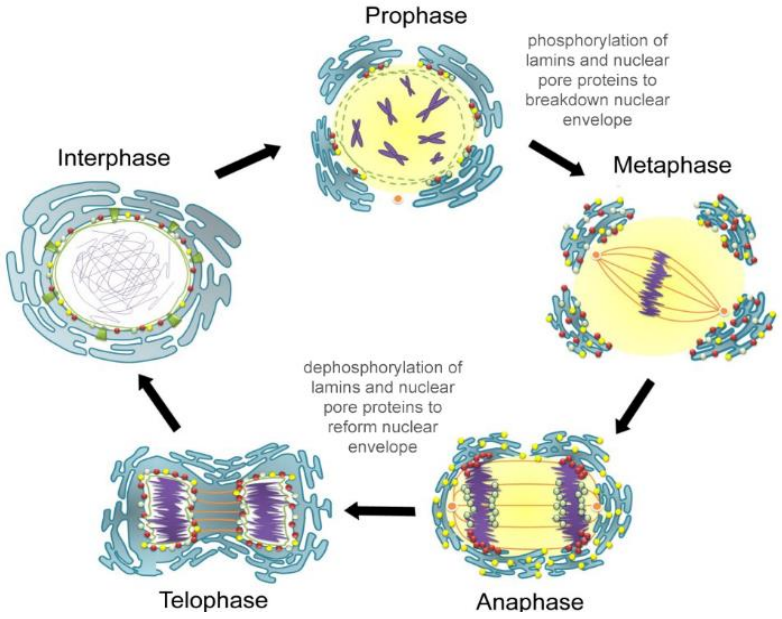

Figure 15 : Depolymerization of laminas and disorganization of the nuclear envelope.

5. Cell cycle checkpoints

Dividing cells go through cycles of cell growth, DNA replication, chromatin condensation, chromosome segregation, and cell division. All these highly complicated processes need to be accurately performed and tightly controlled in order to produce viable offspring. Checkpoints were initially characterized as mechanisms that ensure that a certain process is started only after the previous one has been successfully completed.

Checkpoint pathways monitor progress during cell division so that in the event of an error, the checkpoint is activated to block the cell cycle and activate repair pathways. Intrinsic to this process is that once repair has been achieved, the checkpoint signaling pathway is inactivated and cell cycle progression resumes.

5.1. DNA damage checkpoint

Various forms of DNA damage can be induced in cells by endogenous or exogenous agents. An important part of the cellular response to DNA damage is to stop cell cycle progression when DNA damage is sensed. This mechanism, termed the DNA damage checkpoint, is believed to facilitate DNA repair and prevent genomic instability. The essential role of the checkpoint is highlighted by the fact that genetic mutations of the pathway are often implicated in human diseases.

Phosphatidylinositol-3-OH kinase-like kinases ataxia telangiectasia mutated (ATM), and ataxia telangiectasia and Rad3 related (ATR) are two major signaling networks that are activated in response to DNA damage (Figure 16). By activating checkpoint kinases 1 and 2 (Chk1/2), respectively. ATR and ATM control G1/S, intra-S, and G2/M cell cycle arrest for DNA repair. In addition, the p38 MAPK-MK2 pathway, downstream of ATM and ATR, is activated in response to DNA damage caused by chemotherapy-induced G2/M arrest. The checkpoint effector kinases, Chk1, Chk2, and MK2 have been described as inactivating CDC25 phosphatases, causing CDK/cyclin inactivation and cell cycle arrest.

The upstream ATM responds to double-strand DNA breaks (DSB) while ATR is activated by single-strand DNA breaks (SSB). Activation of ATM–Chk2 and ATR–Chk1 pathways result in the inhibitory phosphorylation of the CDC25 phosphatases. Chk1 and Chk2 target CDC25A for degradation while Chk1-mediated phosphorylation of CDC25B and CDC25C leads to 14-3-3 binding, nuclear exclusion of the phosphatases, and cell cycle arrest. CDC25A normally activates CDK2/cyclin complexes causing S-phase progression while CDC25B/C activates CDK1/cyclin complexes resulting in G2/M transition. By removing inhibitory phosphate groups from specific CDKs, CDC25 phosphatases promote cell cycle progression. In contrast, CDK4/cyclin and CDK6/cyclin complexes control the G1/S checkpoint and are both regulated by p53 and its downstream cyclin-dependent kinase inhibitor p21. ATM and Chk2 activate p53 by promoting its stability. In response to DNA damage, the activation of the p38 MAPK–MK2 pathway is mediated by thousand-and-one amino acid (TAO) kinases, which are activated by ATM and ATR through as-yet-uncharacterized mechanisms. p38 MAPK can be directly activated by UV independently of ATM and ATR. MK2 inhibits CDC25A, CDC25B, and CDC25C phosphatases in a similar fashion to Chk1 and Chk2, resulting in inactivation of CDK1/2-cyclin complexes and cell cycle arrest. Wee1 is another checkpoint kinase downstream of Chk1 that directly phosphorylates CDK1, resulting in G2/M arrest. Through these pathways, cell cycle arrest enables cells to overcome their DNA damage and enhances survival.

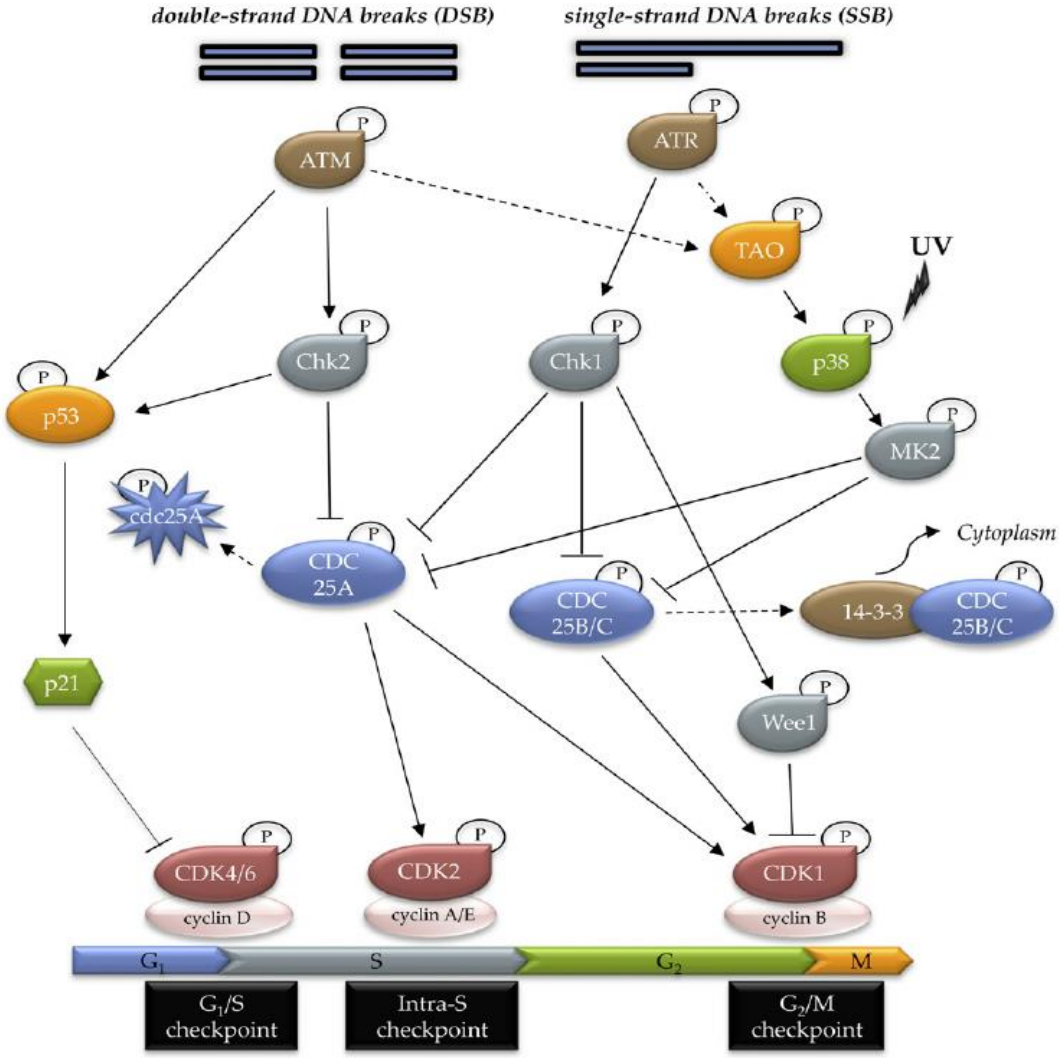

Figure 16: Molecular mechanism of the DNA damage response network.

The integrity of the cell's genome is monitored by the transcription factor p53. In the presence of genomic damage, p53 interrupts cycling to allow time for DNA repair, this is accomplished by p53 inhibition of RB phosphorylation. Normally, in replicating cells, levels of p53 are low or undetectable, these low levels are maintained so that normal replication may continue unimpeded. p53 is negatively regulated by MDM2 (murine double-minute 2) protein; MDM2, reciprocally, is regulated by p53. MDM2 functions at two sites: at the level of the gene it downregulates p53 transcription; it also binds to p53 protein, decreasing p53 activity, and mediating its export from the nucleus, ubiquitination and proteasomal degradation. In the presence of DNA damage, p53 binds to its sequence-specific DNA site; gene induction results in increased p53 protein synthesis. The subsequent phosphorylation of p53 activates the protein, and there is reduced binding and inactivation by MDM2 with doubling of

the p53 half-life. As a result, p53 protein activity may increase a hundred-fold. p53 control of the cell cycle operates through transcriptional upregulation of the CDK inhibitor (CKI) p21, an active inhibitor of CDKs 4, 6, and 2. The inhibition of kinase activity prevents phosphorylation of RB and, as a result, the cell remains in G1 allowing time for DNA repair. When DNA damage exceeds the capacity of the cell for repair, p53 guides the corrupt cell into apoptosis by inducing the expression of the pro-apoptotic protein Bax. Two families of CDK inhibitors are involved in cell cycle regulation. The Cip/Kip family includes the inhibitors p21 and p27; they function at several sites in the cell cycle, targeting CDKs 4, 6, and 2. The second family includes the constitutively expressed INK4 (inhibitors of cyclin dependent kinase 4) genes. The INK4a gene encodes two distinct transcripts, p16INK4a and p19ARF. The CKI p16INK4a specifically inhibits CDK4/6; p19ARF binds to MDM2 and blocks destruction of p53. In response to upregulation of p53, there is an increase in p21 followed by inhibition of CDK4 and CDK6. As a result of the action of these CKIs (p21 and the INK4 proteins), phosphorylation of RB is inhibited and the cell remains in G1.

5.2. DNA replication checkpoint

The maintenance of genome integrity is critical for the faithful replication of the genome during cell division and for protecting cells from accumulation of DNA damage, which if left unrepaired leads to a loss of genetic information, a breakdown in cell function and ultimately cell death and cancer.

Successful DNA replication requires duplicating billions of base pairs of DNA in each cell division cycle rapidly, accurately, and completely. In addition, the epigenetic state of the DNA and chromatin must be re-established. These processes are executed by a large protein machine called the replisome, consisting of polymerases, helicases, nucleases, and ligases that copy the DNA as well as accessory factors that assemble chromatin on the newly synthesized DNA strands. Genetic inheritance is challenged by damaged template DNA, collisions with transcription complexes, difficult-to-replicate DNA sequences, and other sources of replication stress, including oncogene activation .

Eukaryotic cells have evolved mechanisms, usually termed as the replication checkpoint, that monitor the occurrence of replication stress and trigger a cellular response aimed at preserving genome integrity. The replication checkpoint constitutes a specialized branch of the DNA damage checkpoint and it is often referred to as the S phase (or intra-S phase) checkpoint.

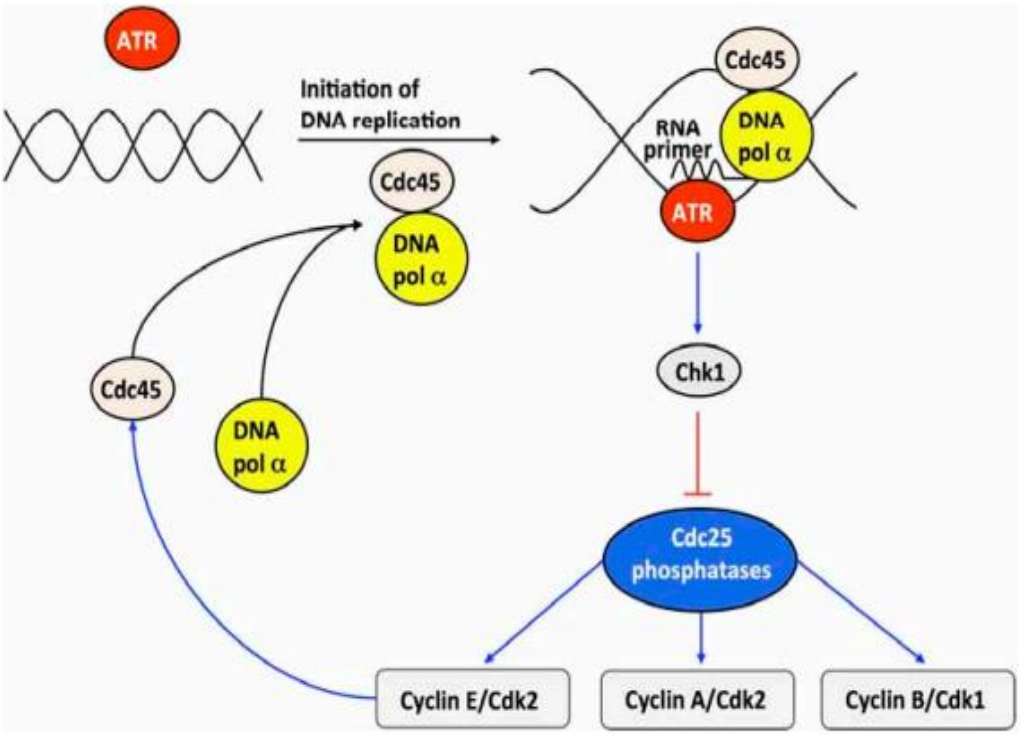

Figure 17: Scheme of the DNA replication checkpoint regulated by kinases ATR and Chk1.

At the G1/S transition, cyclin E/Cdk2 activates, by phosphorylation, the anchor factor Cdc45 that allows DNA polymerase α to bind to DNA. Upon initiation of DNA replication, DNA polymerase α synthesizes an RNA primer, which binds and activates the kinase ATR. The active form of ATR activates, by phosphorylation, the kinase Chk1. Once activated, Chk1 inhibits, by phosphorylation, the phosphatases Cdc25 responsible for the activation of cyclin E/Cdk2, cyclin A/Cdk2, and cyclin B/Cdk1. The inhibition of cyclin E/Cdk2 and cyclin A/Cdk2 during DNA replication creates a checkpoint, which limits the activation of Cdc45 and thereby prevents excessive initiation of DNA synthesis at multiple points of origin of DNA replication. At the end of DNA replication, cyclin E/Cdk2 is further inhibited due to the degradation of cyclin E, brought about by the rise in Skp2, which follows from the inactivation of Cdh1 by cyclin A/Cdk2. Cdc45 will not be active anymore, due to the inactivation of cyclin E/Cdk2, so that the activity of DNA polymerase α will decrease, and so will the concentration of RNA primer and the activity of the kinases ATR and Chk1. Because the ATR/Chk1 checkpoint promotes the inhibition of the phosphatase Cdc25 that activates cyclin B/Cdk1, the resulting inhibition of cyclin B/Cdk1 prevents cells to enter into mitosis as long as DNA replication is not completed.

This checkpoint is mediated by the ATR/Chk1 pathway, which inhibits the phosphatases Cdc25 that activate Cdk2 and Cdk1. At the G1/S transition, cyclin E/Cdk2 activates by phosphorylation the anchor factor Cdc45, which permits the binding of DNA polymerase α to DNA and the initiation of DNA replication (Figure 17). The kinase ATR is activated upon binding the RNA primer synthesized by DNA polymerase α. ATR phosphorylates, and thereby activates, the kinase Chk1. Once activated, Chk1 inhibits the Cdc25 phosphatases; this inhibition blocks cell cycle progression by preventing the activation of Cdk2 and Cdk1 as long as DNA replication proceeds. Finally the decrease in Cdk2 activity, inherent to the oscillatory dynamics of the Cdk network, inhibits DNA polymerase at the end of the S phase. The subsequent inhibition of ATR and Chk1 relaxes the inhibition of the phosphatases Cdc25 and thereby permits the rise in the activity of cyclin B/Cdk1 that will elicit the G2/M transition.

5.3. Spindle assembly checkpoint

The spindle checkpoint protects cells from chromosome missegregation caused by mitotic errors. When the checkpoint is activated by defects in kinetochore-microtubule attachment, it arrests the cell cycle by inhibiting the anaphase-promoting complex (APC).

After nuclear envelope breakdown at the prophase to prometaphase transition, each of the two kinetochores formed at the centromeric regions of paired sister chromatids initiates attachment to spindle microtubules, with the goal of achieving bi-orientation with sister kinetochores oriented toward opposite poles of the spindle. However, in response to unattached kinetochores, the **spindle assembly checkpoint** (SAC) is activated, through hierarchical recruitment of SAC proteins, including MAD2, BUB3, BUBR1, and CENP-E, at the unattached kinetochores. Consequently, MAD2, BUBR1, and BUB3 form a complex with CDC20, known as the mitotic checkpoint complex (MCC) (Figure 18). This way, CDC20 is sequestered and prevented from activating the anaphase-promoting complex/cyclosome (APC/C), which is a ubiquitin E3 ligase that promotes proteolysis of securin and cyclin B and subsequent mitotic exit.

Once all the chromosomes are aligned with their kinetochores attached to the spindle (metaphase), generation of the MCC ceases, allowing Cdc20 to activate the APC/C, leading to the ubiquitylation and degradation of securin and cyclin B1. Degradation of securin liberates separase which in turn cleaves the Scc1 kleisin subunit of the cohesin ring structure; this opens the ring, allowing sister chromatids to separate (anaphase). Meanwhile, degradation of cyclin B1 inactivates Cdk1, leading to mitotic exit.

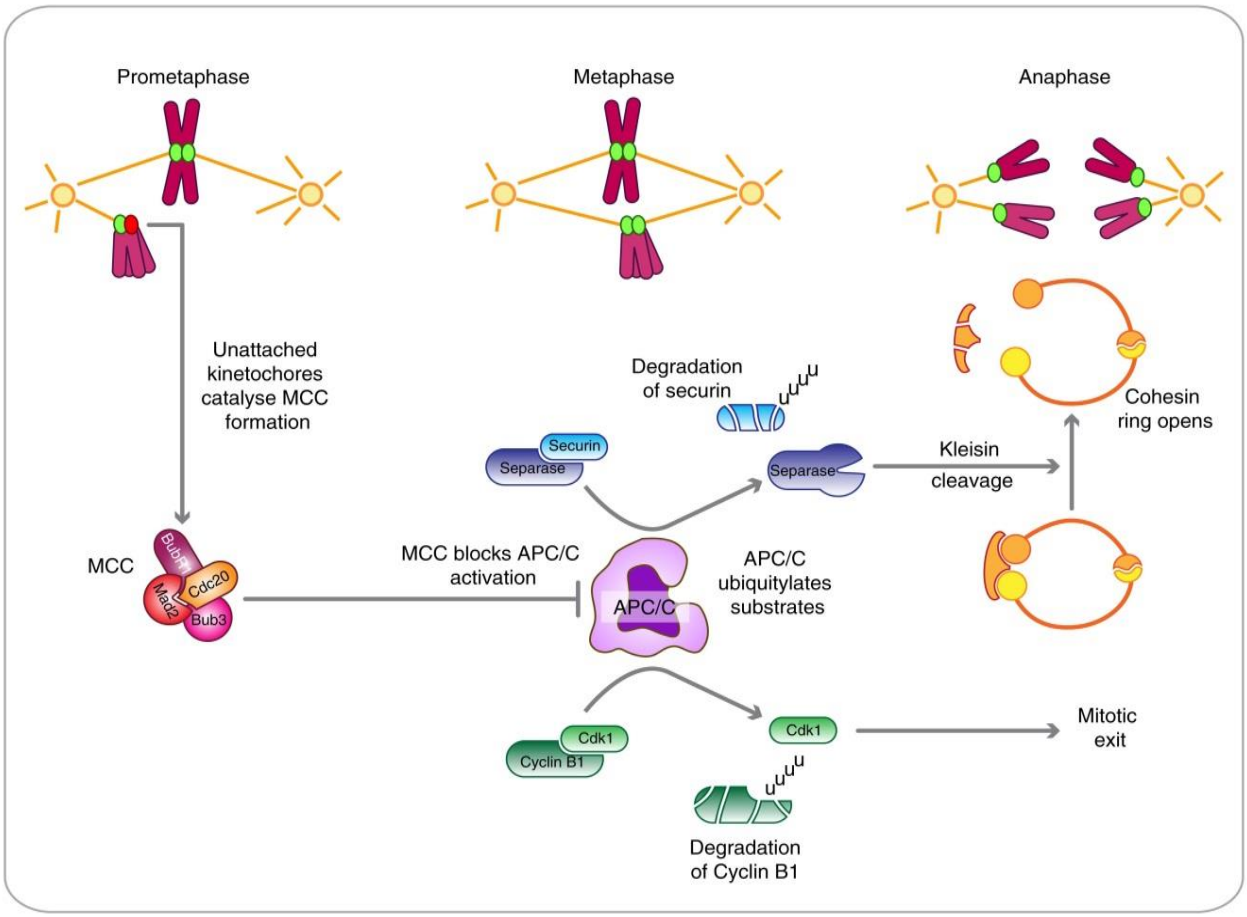

Figure 18: Spindle assembly checkpoint mechanism.

During the early stages of mitosis (prometaphase), unattached kinetochores catalyse the formation of the mitotic checkpoint complex (MCC) composed of BubR1, Bub3, Mad2 and Cdc20, leading to inhibition of the APC/C. Once all the chromosomes are aligned with their kinetochores attached to the spindle (metaphase), generation of the MCC ceases, allowing Cdc20 to activate the APC/C, leading to the ubiquitylation and degradation of securin and cyclin B1. Degradation of securin liberates separase which in turn cleaves the Scc1 kleisin subunit of the cohesin ring structure; this opens the ring, allowing sister chromatids to separate (anaphase). Meanwhile, degradation of cyclin B1 inactivates Cdk1, leading to mitotic exit.

6. Apoptosis

6.1. Introduction

Apoptosis or programmed cell death is an ordered and orchestrated cellular process that occurs in physiological and pathological conditions. Apoptosis occurs normally during development and aging and as a homeostatic mechanism to maintain cell populations in tissues. Apoptosis is considered a vital component of various processes including normal cell turnover, proper development and functioning of the immune system, hormone-dependent atrophy, embryonic development and chemical-induced cell death. Apoptosis also occurs when cells are damaged by disease or noxious agents. Inappropriate apoptosis is a factor in many human conditions including neurodegenerative diseases, ischemic damage, autoimmune disorders and many types of cancer.

This program involves the sequential activation of particular proteases called caspases, which are cysteine proteases.

Caspases exist in all cells in the form of inactive precursors (procaspases) whose activation of a first group of initiator caspases leads to the sequential activation of effector caspases, within a proteolytic cascade.

The activation process can be initiated by various types of intra- and extra-cellular signals: the extrinsic pathway of death domain receptors (Fas, TNF), and the intrinsic mitochondrial pathway.

6.2. Morphological conditions in apoptosis

Morphologically the presence of apoptosis renders the cell with shrinkage and pyknosis, this is characterized by DNA fragmentation, chromatin condensation and the compacting of the cytoplasm. The blebbing of the plasma membrane is followed by these conditions which causes the nucleus to break (karyorrhexis). This further causes the cells to detach from its surrounding tissues and to be separated into cell fragments with the cytoplasm embedded with tightly packed organelles. Next, this compact bodies referred to as apoptotic bodies assume the process of budding. Finally, the release of cell surface markers (phosphatidylserine) from the cell membrane enhance

some cells such macrophages and parenchyma to phagocytized these bodies for further degradation and also to prevent secondary necrosis (Figure 19).

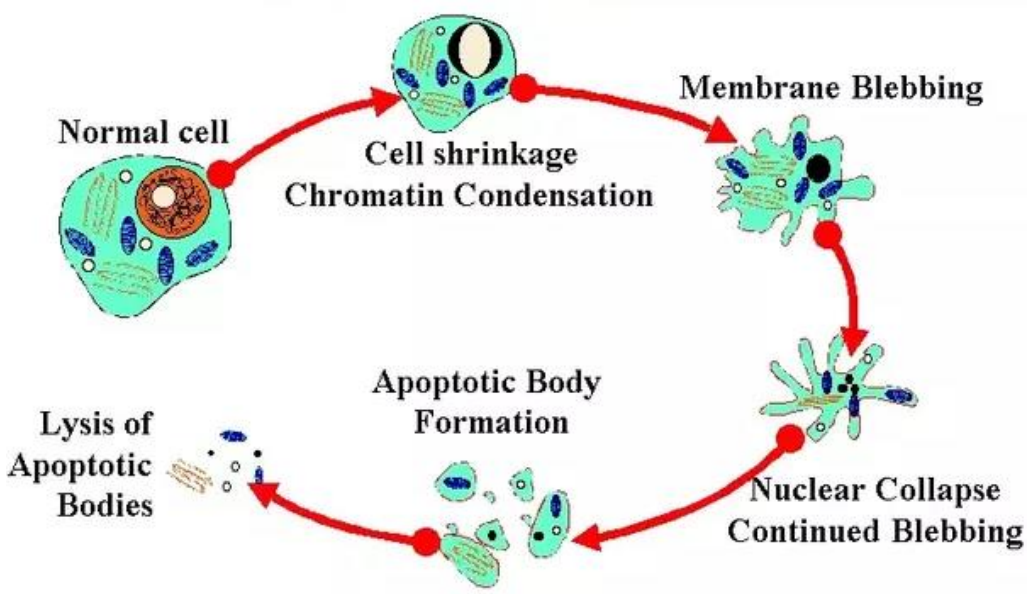

Figure 19: Morphology of apoptosis.

6.3. Biochemical changes in apoptosis

Broadly, three main types of biochemical changes can be observed in apoptosis: activation of caspases, DNA and protein breakdown and membrane changes and recognition by phagocytic cells.

Early in apoptosis, there is expression of phosphatidylserine (PS) in the outer layers of the cell membrane, which has been "flipped out" from the inner layers. This allows early recognition of dead cells by macrophages, resulting in phagocytosis without the release of pro-inflammatory cellular components.

Another specific feature of apoptosis is the activation of a group of enzymes belonging to the cysteine protease family named caspases. The caspase which is a cysteine protease responsible for the cleavage of aspartic residue upon its activation, thus a cysteine dependent aspartate-specific protease. These are inactive proenzyme which once activated, it in tend activates other pro-caspase to initiate a protease cascade and the execution of a number of cell death process. Activated caspases cleave many vital cellular proteins and break up the nuclear scaffold and cytoskeleton. They also activate DNAase, which further degrade nuclear DNA into large 50 to 300

kilobase pieces. Later, there is internucleosomal cleavage of DNA into oligonucleosomes in multiples of 180 to 200 base pairs by endonucleases.

6.4. Structure and activation mechanism of Caspases

At least 14 distinct mammalian caspases have been identified. Caspases involved in apoptosis are generally divided into two categories, the initiator caspases, which include caspase-2, -8, -9, and -10, and the effector caspases, which include caspase-3, -6, and -7.

The initiator caspases are activated by formation of caspase-activating complexes, which function as a platform to recruit caspases, providing proximity for self-activation. Well-known initiator caspase-activating complexes include: DISC (Death Inducing Signaling Complex), which activates caspases-8 and 10; Apoptosome, which activates caspase-9; and PIDDosome, which activates caspase-2 (Figure 20).

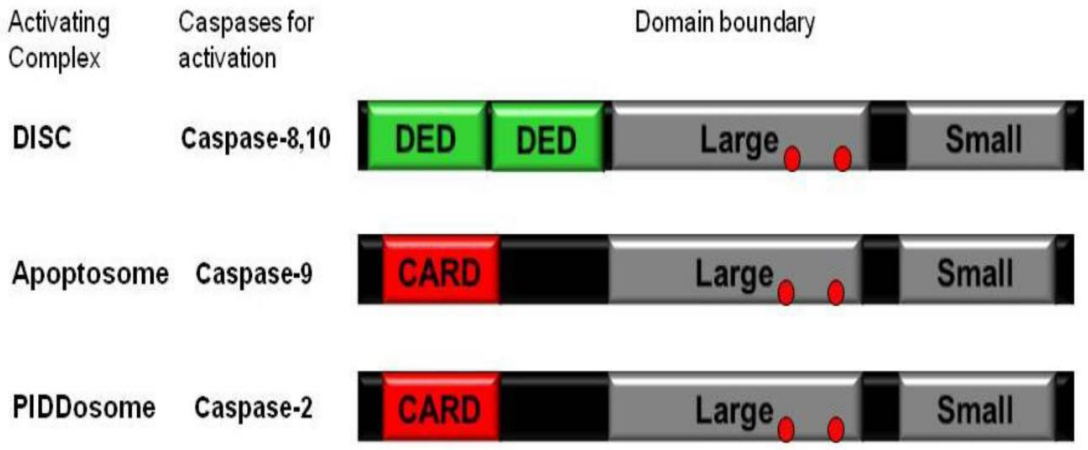

Figure 20: Initiator caspase-activating complexes.

An initiator caspase is characterized by an extended N-terminal prodomain (>90 amino acids) important for its function, whereas an effector caspase contains 20–30 residues in its prodomain sequence. All caspases are produced in cells as catalytically inactive zymogens and must undergo proteolytic activation during apoptosis. The activation of an effector caspase (such as caspase-3 or -7) is performed by an initiator caspase (such as caspase-9) through cleavage at specific internal Asp residues that separate the large and small subunits. The initiator caspases, however, are

autoactivated. As the activation of an initiator caspase in cells inevitably triggers a cascade of downstream caspase activation, it is tightly regulated and often requires the assembly of a multicomponent complex under apoptotic conditions.

Caspases are synthesized as single-chain zymogens and require a highly regulated process for their activation (Figure 21A). Fully activated caspases are dimeric with two large subunits and two small subunits in general (Figure 21B). Both cleavage and dimerization are important to the integrity of the caspase active sites and therefore are required for caspase activation. Cleavage and dimerization-dependent caspase activation is still a question under debate because several study showed that cleavage and dimerization of caspase-9 were not required for its activation.

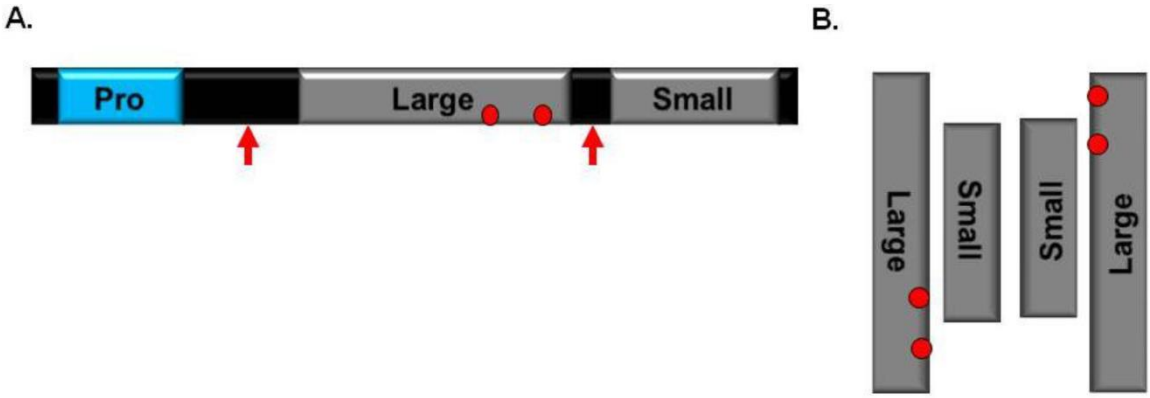

Figure 21: Proteolytic caspase activation. (***A****) Domain boundary of initiator caspase. Red arrows indicate proteolytic sites. Red circle indicates the catalytic sites; (****B****) Activated caspases. Red circles indicates the catalytic sites; The pro-caspase is inactive and its typical structure contains three parts: a pro-domain, a large subunit, and a small subunit. The length of pro-domain vary among caspases and some of that has death effector domain feature (DED) or caspse recriutment domain feature (CARD), pro-enzyme is cleaved at caspase cleavage sequences (Aps-X), two large and two small subunits combine to form the active tetrameric enzyme.*

6.5. Mechanisms of apoptosis

Caspases are central to the mechanism of apoptosis as they are both the initiators and executioners. There are three pathways by which caspases can be activated. The two commonly described initiation pathways are the intrinsic and extrinsic (or death receptor) pathways of apoptosis (Figure 22).

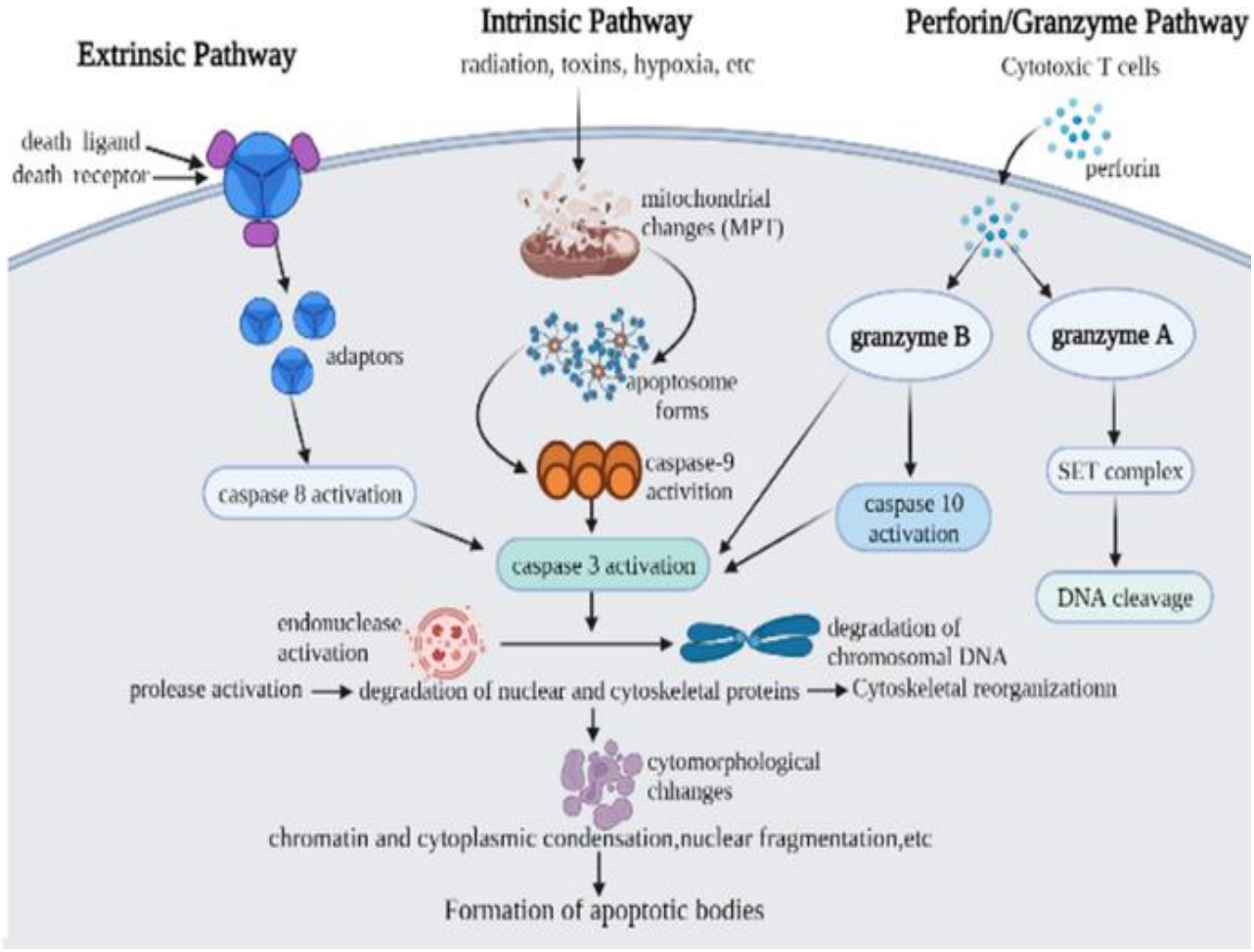

Figure 22: Intrinsic and extrinsic pathways of apoptosis.

The intrinsic can further be categorized into two which is the intrinsic mitochondrial pathway and the intrinsic endoplasmic reticulum pathway.

In the intrinsic pathway, the cell kills itself because it senses cell stress, while in the extrinsic pathway the cell is instructed to kill itself through signal transduction stimulators from other cells.

There is an additional pathway that involves T-cell mediated cytotoxicity and perforin-granzyme dependent killing of the cell. In this pathway, apoptosis is induced via either Granzyme B or Granzyme A.

The extrinsic, intrinsic, and granzyme B pathways converge on the same terminal, or execution pathway. This pathway is initiated by the cleavage of caspase-3 and results in DNA fragmentation, degradation of cytoskeletal and nuclear proteins, crosslinking of proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors and finally uptake by phagocytic cells. The granzyme A pathway activates a parallel, caspase-independent cell death pathway via single stranded DNA damage.

6.5.1. Extrinsic pathway (death receptor)

Apoptosis is induced by this pathway through a mediated interaction of death ligands with death receptors. Accumulating evidence suggest that these death receptors are members of tumor necrosis factor (TNF) which includes the TNF, Fas ligands (Fas-l) and TNF -related apoptosis inducing ligand (TRAIL).

In the process, the death receptors (Fas receptor, TNF receptors) bind to death ligands (Fas ligands, TNF ligands) to allows the binding of the death domain / adapter Protein (Fas -associated Death Domain (FADD), TNF receptor associated death domain (TRDD)). The binding of the death domain /adapter protein to receptor ligands complex allows the binding of an initiator caspase 8 or 10 through its death effector domain (DED) to form an activated complex called Death inducing signaling complex (DISC). Furthermore, the binding and activation allow the caspase 8 to relay the death signal to an execution caspase to bring about apoptosis (Figure 23).

Death receptor mediated apoptosis can be inhibited by a protein called c-FLIP which will bind to FADD and caspase-8, rendering them ineffective. Another point of potential apoptosis regulation involves a protein called Toso, which has been shown to block Fas-induced apoptosis in T cells via inhibition of caspase-8 processing.

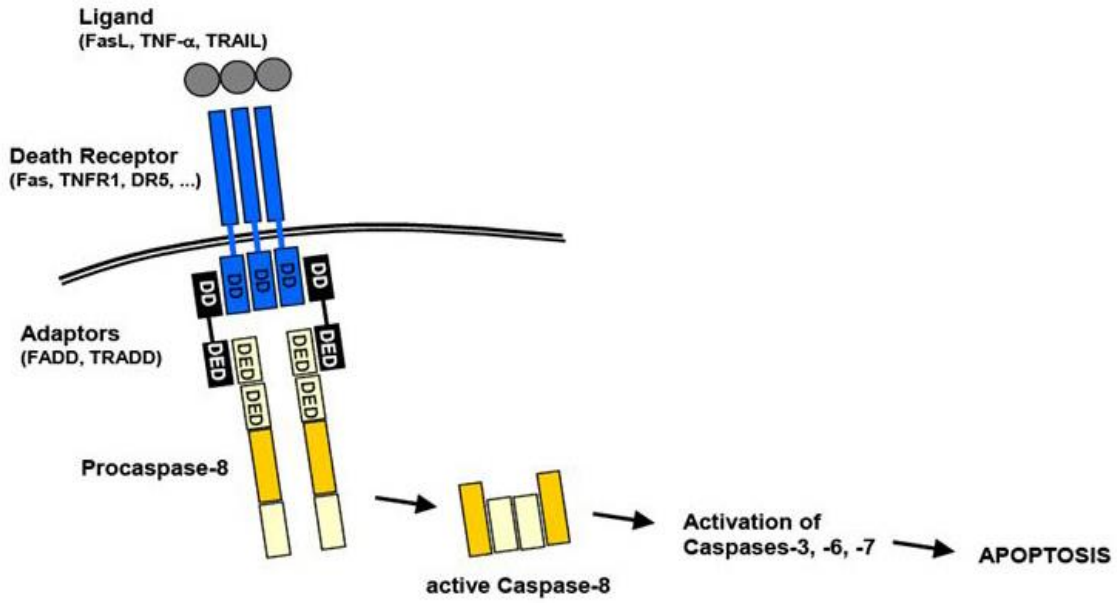

Figure 23: Extrinsic pathway (death receptor).

6.5.2. Intrinsic mitochondrial pathway

The intrinsic mitochondrial pathway As its name implies, the intrinsic pathway is initiated within the cell. Internal stimuli such as irreparable genetic damage, hypoxia, extremely high concentrations of cytosolic Ca2+ and severe oxidative stress are some triggers of the initiation of the intrinsic mitochondrial pathway. Regardless of the stimuli, this pathway is the result of increased mitochondrial permeability and the release of pro-apoptotic molecules such as cytochrome-c into the cytoplasm.

The release of cytochrome C helps to assemble the Apoptosome, which contains Apaf-1, caspase-9, and cytochrome C (Figure 24). Apaf-1, the central component of the apoptosome, contains an N-terminal CARD, an expanded nucleotide-binding domain and a C-terminal WD40 Int. Apaf-1 CARD is responsible for the interaction with the Caspasae-9 CARD, which is essential to the recruitment and the activation of Caspase-9. Then, the initiator caspasa-9 cleaves and activates execution caspases, such as Caspase-3 and caspasae-7, causing cell death (Figure 25).

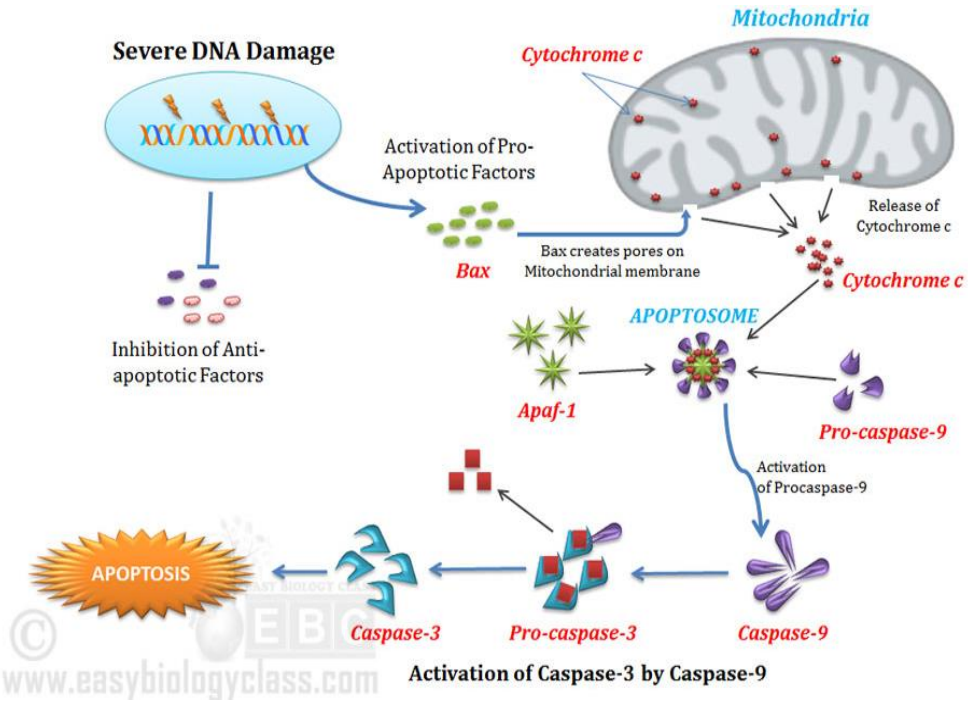

Figure 24: Intrinsic mitochondrial pathway.

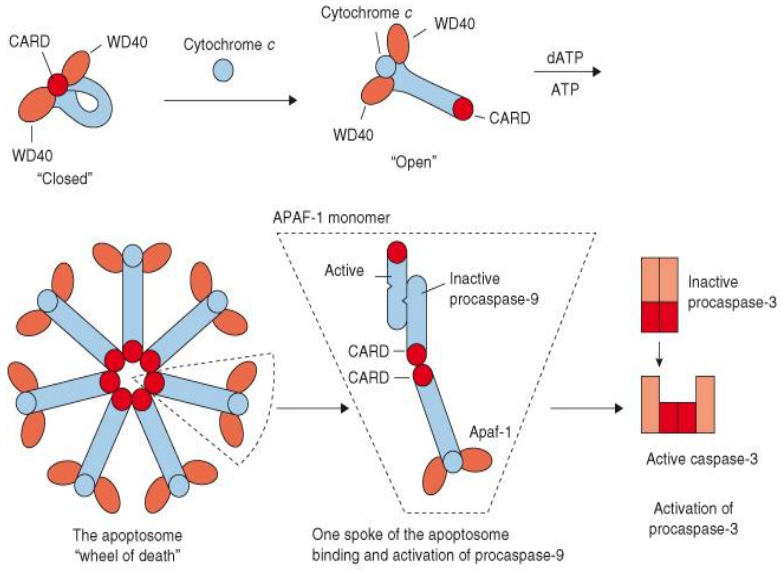

Figure 25: Formation of the apoptosome. *In an individual APAF-1 molecule, two groups of WD40 repeats in the C-terminal region are thought to keep the protein inactive until cytochrome c engages the repeats. Association with cytochrome c causes APAF-1 to convert from a "closed" conformation to a more "open" one.. This binding triggers formation of the active seven-span symmetrical "wheel of death" – the apoptosome – via interaction among the N-terminal caspase recruitment domains (CARD) of the individual APAF-1 molecules. The apoptosome subsequently recruits procaspase-9 into its central hub through CARD–CARD domain interaction between procaspase-9 and APAF-1 molecules. An inactive procaspase-9 monomer on one "spoke" of the apoptosome is thought to recruit another monomer to create a dimer with a single active site. This active caspase activates downstream executioner caspases, such as caspase-3 and caspase-7.*

- **Regulation of the intrinsic mitochondrial pathway**

  The intrinsic mitochondrial pathway is closely regulated by a group of proteins belonging to the Bcl-2 (B-cell lymphoma 2) family.

  The Bcl-2 proteins control cell apoptosis by modulating the mitochondrial outer membrane permeabilization (MOMP) via protein-protein interactions (PPIs) between the pro and anti-apoptotic proteins. . It is not the absolute quantity but rather the balance between the pro- and anti-apoptotic proteins that determines whether apoptosis would be initiated (Figure 26).

  Anti-apoptotic BCL-2 proteins, including BCL-XL, MCL-1, BCL-W, and BFL-1/A1, inhibit MOMP and all have multiple BCL-2 homology (BH) domains. Depending on the structures and the functions they involve in apoptosis regulation, pro-apoptotic BCL-2 proteins can be divided into two subgroups, including BH3-only and multiple BH pore-forming proteins. BH3-only proteins, such as BIK, BIM, BID, BAD, BMF, HRK, NOXA, and PUMA, contain a single BCL-2 BH3 domain, while pore-forming BCL-2 proteins including BAX and BAK contain multiple BH domains.

  Anti-apoptotic BCL-2 proteins interact with BAX/BAK and prevent them from homo-oligomerization and subsequent pore formation in mitochondrial outer membrane. Upon the stimulation of apoptotic signals, the BH3-only proteins promote apoptosis by directly activating BAX and BAK and/or displacing them from the anti-apoptotic partners. Subsequently, BAK/BAX facilitates MOMP via homo-oligomerization and pore formation in the mitochondrial outer membrane, which causes the efflux of cytochrome c from mitochondria into the cytoplasm. Once released in the cytoplasm, cytochrome c binds with apoptotic protease activating factor-1 (APAF-1), which stimulates caspase 9 to activate the effector caspases and the eventual induction of apoptosis.

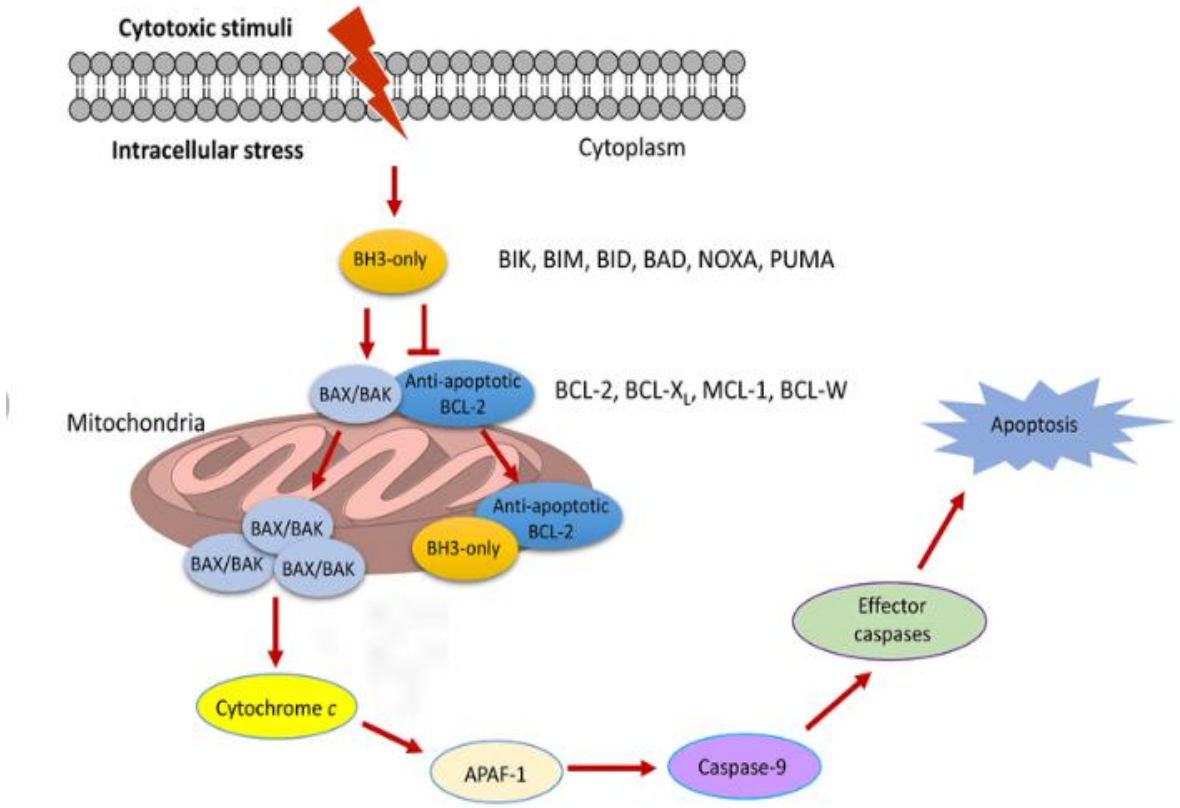

Figure 26: BCL-2 family proteins regulate mitochondria-mediated intrinsic apoptosis pathway.

6.5.3. Caspase-2 Signaling Platform

The PIDDosome is considered to be the main signaling platform for caspase-2 activation in response to DNA damage and cytokinesis failure

The serine/threonine kinase ATM (ataxia telangiectasia mutated) has been shown to directly promote PIDDosome formation induced by DNA damage. ATM phosphorylates Thr788 in the death domain (DD) of PIDD1, leading to conformational changes and allowing binding with RAIDD through DD interactions. More recently, the phosphoprotein nucleophosmin (NPM1) has been shown to control PIDDosome formation specifically in the nucleolus, which activates caspase-2 in response to DNA damage. The authors also demonstrated a RAIDD-dependent, but PIDD1-independent, platform for caspase-2 activation in the cytoplasm, while both RAIDD and PIDD1 are involved in a nucleolar platform.

Caspase-2 was also shown to be activated in DISC, which includes CD95 (FAS/APO-1) and TNFR.

For caspase-2 activation, the PIDDosome must be formed. The PIDDosome is composed of three proteins, p53-induced protein with a death domain (PIDD), RIP-associated Ich-1/Ced-3 homologous protein with a death domain (RAIDD) and caspase-2 . PIDD contains 910, 2 ZU-5 domains and a C-terminal death domain (DD). PIDD can be cleaved into shorter fragments generating a PIDD-N fragment of 48 kD, a PIDD-C fragment of 51 kD and a PIDD-CC fragment of 37 kD (Figure 27).

Figure 27: The cleavage fragments resulting from PIDD autoprocessing are indicated.

Auto-cleavage of PIDD determines the downstream signaling events. The PIDD-C fragment mediates activation of NFκB via the recruitment of RIP1 and NEMO, while PIDD-CC causes caspase-2 activation, which leads to apoptosis. RAIDD is an adaptor protein that contains both an N-terminal caspase recruitment domain (CARD) and a C-terminal DD. Caspase-2 possesses an N-terminal CARD pro-domain. Both CARD and DD with the death effector domain (DED) and the Pyrin domain (PYD) belong to the DD superfamily, which is a well-known protein interaction module. Many DD superfamilies contain proteins that participate in the formation of large molecular machines involved in activation of signaling enzymes, such as caspases and kinases. For the PIDDosome assembly, RAIDD and PIDD interact with each other via their DDs, while RAIDD and caspase-2 interact with each other via their CARDs (Figure 28).

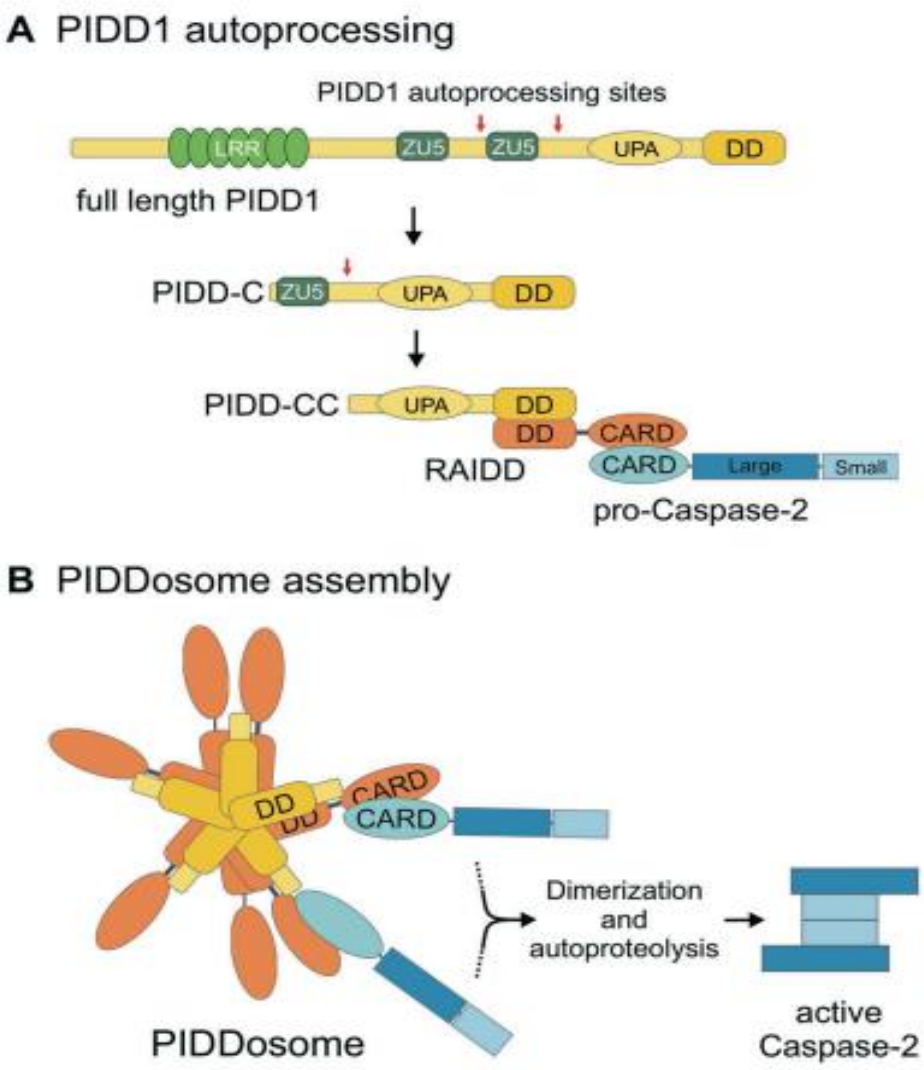

Figure 28: PIDD1 autoprocessing and caspase-2 activation.

(A) Fulllength PIDD1 undergoes autoprocessing at specific residues S446 and S588 (red arrows) to generate two C-terminal fragments with distinct functions, PIDD-C and PIDD-CC. The former has been implicated in NFκB signaling while the latter interacts with the death domain (DD) of RAIDD to form the PIDDosome. The caspase activation and recruitment domain (CARD) of RAIDD interacts with procaspase-2. (B) The core of the PIDDosome complex is formed by 5:5 PIDD-CC: RAIDD molecules interact via the DDs, and two additional RAIDD entities are placed on top protruding in diametric orientation (based on the protein database structure 2OF5). Binding of RAIDD to procaspase-2 promotes proximity-induced dimerization and autocleavage of caspase-2, critical for its activation.

6.5.4. Intrinsic Pathway via Endoplasmic Reticulum Stress-Induced Apoptosis

The main stimulus of this pathway is the misfolding of proteins and their subsequent accumulation in the endoplasmic reticulum (ER) (Figure 29). Once the misfolded proteins reach a critical concentration, ER membrane sensors such as protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring protein 1 (IRE1a) and activating transcription factor 6 (ATF6) are activated.

- **ERK Signaling pathway**

  In different cell types it has been demonstrated that cell death is induced via the PERK signaling pathway under chronic stress conditions. The key molecule for initiating cell death is C/EBP homologous protein (CHOP), also named as growth arrest and DNA-damage-inducible 153 (GADD153). The expression of CHOPs is increased by ATF4. PERK activation induces eIF2α phosphorylation which in turn increases the selective transcription of ATF4 that increases CHOP level. Pro-apoptotic proteins such as GADD34, ERO1α (ER oxidase 1 alpha) and BH3-only proteins (BIM, PUMA and NOXA) expressions is increased by CHOP. BIM, PUMA and NOXA induction cause cytochrome-c release from mitochondria via BAX and BAK activation. PERK signaling pathway initiates mitochondrial apoptotic pathway.

- **ATF6 signaling pathway**

  Under non-stress conditions, ATF6 resides in the endoplasmic reticulum membrane in the form of zymogen, via its hydrophobic sequence. The ATF6 activation process during ER stress is different from the PERK and IRE1 activation processes. After GRP78 (cChaperone protein) releases from ATF6, it translocates to the Golgi apparatus from the ER, where it undergoes cleavage by site-1 and site-2 (S1P and S2P) proteases and then relocate to the nucleus with the nuclear localization signal, inducing transcriptional expression of the endoplasmic reticulum stress gene in the nucleus.. GRP78, GRP94, protein disulfide isomerase, CHOP and XBP1 are some of the targets of ATF6.

- **IRE1 signaling pathway**

IRE1 is another protein kinase located on the endoplasmic reticulum membrane. The active IRE1a can recruit the adapter TRAF2 activating apoptosis signal-regulating kinase 1/ MAP3K5 (ASK1) and its downstream target c-Jun Nterminal kinase 1 (JNK/MAPK8/SAPK1). JNK regulates and activates apoptotic pathways and can also participate in necrosis in response to ER stressors.

Generally, IRE1 release has a strong pro-survival effect during stress conditions via UPR; however, long term active IRE induces kinase activities through the c-Jun N-terminal kinase (JNK) pathway and recruitment of TNF-receptor-associated factor 2 molecule (TRAF2). The IRE1-TRAF2 complex causes recruitment of apoptosis-signal-regulating kinase (ASK1) which in turn activates MAPKs JNK and p38. JNK activation associated with Bcl2 family members' regulation in different stress conditions. During ER stress JNK phosphorylates the Bcl2 and inhibits its anti-apoptotic function, however while JNK phosphorylates Bcl-2 homology domain 3 (BH3) and Bim, their pro-apoptotic features gets activated. As an important initiator of apoptosis, IRE1 is the last resorts for the ER stress regulated UPR after PERK and ATF6. IRE1 is the top step for modulation of pro-surviving or cell-death in the cell via ASK1 and JNK.

On the other hand, activated TRAF-2 simultaneously activates Caspase12 and initiates caspase cascade to mediate apoptosis. In addition, IRE1 also has ribonuclease activity which cleaves XBP1 mRNA to promotes the maturation of XBP1 mRNA and enhances the transcriptional expression of molecular chaperone protein and CHOP, thereby promoting apoptosis.

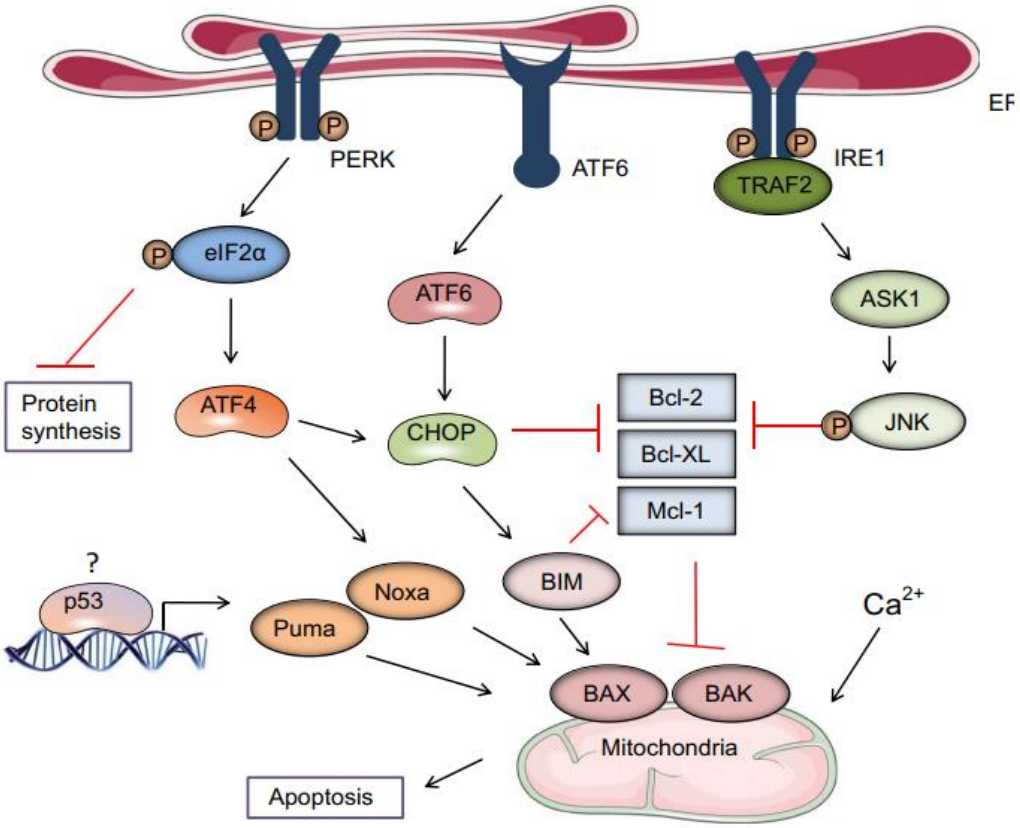

Figure 29: ER stress-induced cell death. *Under ER stress, PERK is activated and phosphorylates and inactivates eIF2a. This results in the selective induction of ATF4 and its downstream proteins CHOP and Noxa, resulting in cell death. CHOP, which can also be induced by ATF6, induces Bim and inhibits Bcl-2. It is still not clear how p53 is induced under ER stress and induces Noxa and Puma, resulting in apoptosis. IRE1a can recruit TRAF2, which activates ASK1 and its downstream target JNK. JNK can induce apoptosis by inhibiting antiapoptotic proteins such as Bcl-2 and Bcl-xL.*

6.5.5. Perforin/Granzyme Pathway

The granzymes (granule enzymes) are a family of highly homologous serine proteases contained in cytotoxic granules of innate and adaptive immune killer cells. Their major job is to induce cell death to eliminate viruses and tumor cells. The granzymes may also play a role in immune regulation by controlling the survival of activated lymphocytes and may also regulate inflammation by acting on extracellular substrates.

A key protein in the cytotoxic granule is perforin. Classically, perforin is known to form a pore in cell membranes, allowing passage of granzymes into the cell inducing apoptosis. The perforin/granzyme pathway (Figure 30) can induce apoptosis via either granzyme B or granzyme A, family of serine proteases. Granzyme A pathway causes cell death via single stranded DNA damage which is caspase independent where as granzyme B mediated cascade is caspase dependant leading to activation of caspase 3 either directly or through caspase 10.

Granzyme B also utilizes the mitochondrial pathway for amplification of the death signal by specific cleavage of Bid. The interaction of Bid with pro-apoptotic Bcl-2 family proteins (Bax/Bak) result in the escape of mitochondrial mediators such as cyt c into the cytoplasm which later triggers the activation of the caspase dependant pathway.

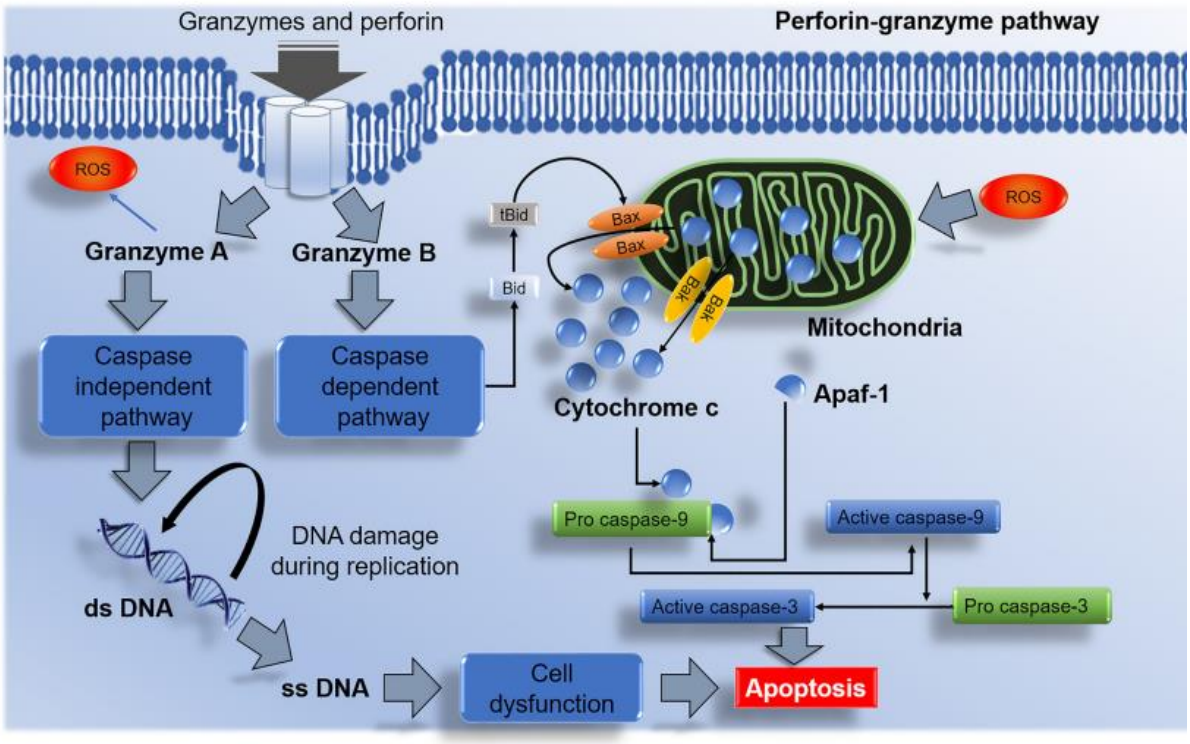

Figure 30: Perforin-granzyme NK cells mediated apoptotic pathway.

Granzyme A activates the caspase-independent pathway and results in DNA damage and apoptosis. Granzyme B initiates the caspase-dependent pathway via Bax/Bak interactions with the mitochondria and releases cytochrome c. The discharge of cytochrome c from the mitochondrion leads to apoptosome formation with the aid from apoptotic activator protease factor 1 (Apaf-1) and pro caspase-9 which in the process gets activated and initiates the stimulation effector caspase-3 and induces apoptosis.

6.6. Final common pathway of apoptosis

The extrinsic, intrinsic and perforin pathways all end at a common point leading to the commencement of the final pathway of apoptosis (Figure 31). Caspase 3, caspase 6, and caspase 7 function as effector or "executioner" caspases, that ultimately cause the morphological and biochemical changes seen in apoptotic cells. Caspase is considered to be the most important of the executioner caspases and is activated by any of the initiator caspases (caspase 8, caspase 9, or caspase 10). Caspase 3 specifically activates the endonuclease CAD (caspase activated DNAse I). In proliferating cells CAD is complexed with its inhibitor, ICAD (inhibitor of caspase activated DNAse I) but in apoptotic cells, activated caspase 3 cleaves ICAD to release CAD. CAD then degrades chromosomal DNA within the nucleus and causes chromatin condensation, results in DNA fragmentation finally cell death and uptake by phagocytic cells.

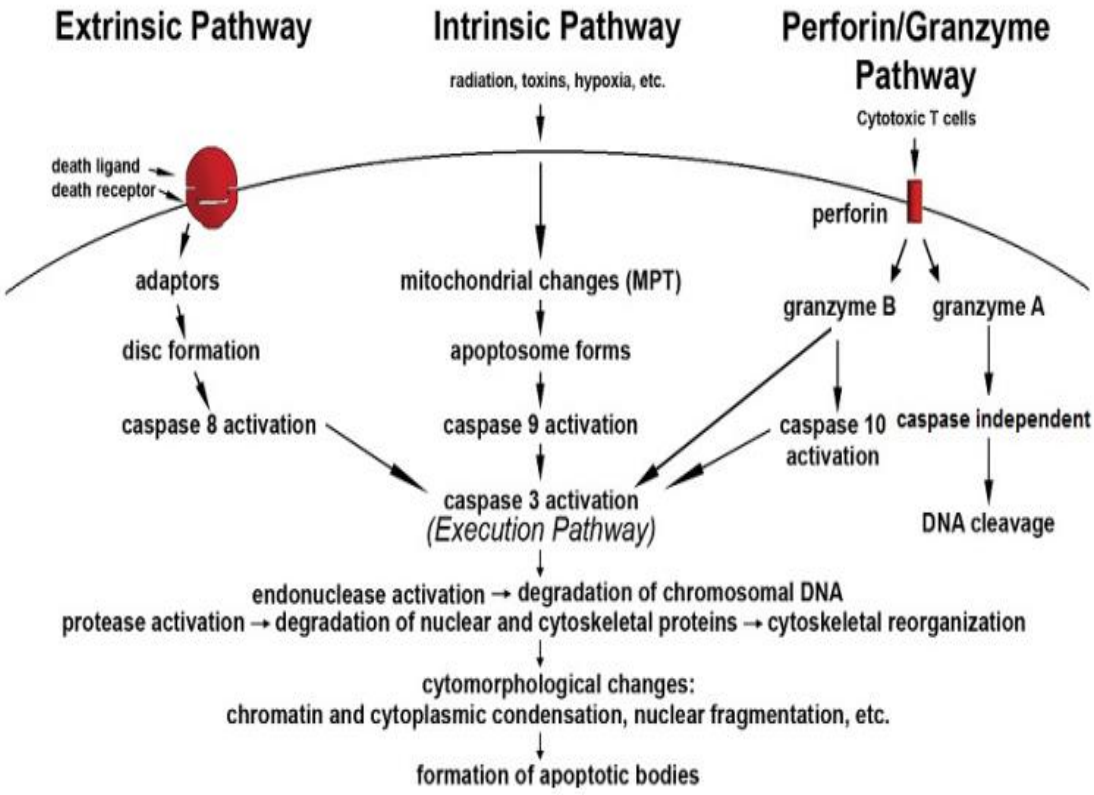

Figure 31: Extrinsic, intrinsic and perforin/granzyme pathway leading common pathway to cell death.

7. Benign and malignant tumors.

7.1. Introduction

Aberrancy in cell cycle progression is one of the fundamental mechanisms underlying tumorigenesis, making regulators of the cell cycle machinery rational anti-cancer therapeutic targets. Growing body of evidence indicates that the cell cycle regulatory pathway integrates into other hallmarks of cancer, including metabolism remodeling and immune escaping. Thus, therapies against cell cycle machinery components can not only repress the division of cancer cells, but also reverse cancer metabolism and restore cancer immune surveillance.

7.2. Definition

A tumor is a pathologic disturbance of cell growth, characterized by excessive and abnormal proliferation of cells. Tumors are abnormal mass of tissue which may be solid or fluid filled. When the growth of tumor cells confined to the site of origin and are of normal physicality they are concluded as benign tumors. When the cells are abnormal and can grow uncontrollably, they are concluded as cancerous cells; malignant tumor. Tumors are also called as 'NEOPLASM'.

7.3. Cell cycle and cancer

Loss of control of the cell cycle is usually a critical step in cancer development. Cells become abnormal and processes regulating normal cell division are disrupted. Cancer cells are caught in an unregulated cell cycle. Most cancers aren't a result of a single event or factor. A number of factors are required for a normal cell to evolve into a cancerous cell and these factors include both environment and heredity. There are four main types of genetic change seen in cancer: spontaneous mutagenesis; environmentally-induced mutagenesis (causative agents include chemicals, radiations and viruses); environmentally-induced mutagenesis, but with genetic predisposition; and change due to hereditary factors.

7.4. Types of cancer

Both benign and malignant tumors are classified according to the type of cell from which they arise. Most cancers fall into one of three main groups: carcinomas,

sarcomas, and leukemias or lymphomas. Carcinomas, which include approximately 90% of human cancers, are malignancies of epithelial cells. Sarcomas, which are rare in humans, are solid tumors of connective tissues, such as muscle, bone, cartilage, and fibrous tissue. Leukemias and lymphomas, which account for approximately 8% of human malignancies, arise from the blood-forming cells and from cells of the immune system, respectively. Tumors are further classified according to tissue of origin (e.g., lung or breast carcinomas) and the type of cell involved. For example, fibrosarcomas arise from fibroblasts, and erythroid leukemias from precursors of erythrocytes (red blood cells.

7.5. Cancer and normal cells

Cancer can be defined as a disease in which a group of abnormal cells grows uncontrollably by forgetting the normal rule of cell division. Normal cells are constantly bound by signals that dictate whether the cell should divide, differentiate into another cell, or die. Cancer cell develops a degree of autonomy from these signals, resulting in uncontrolled growth and proliferation. If this proliferation is allowed to continue and spread, it can be fatal (Table III).

Modification of a normal cell into a cancerous cell is perhaps not such a critical event in the genesis of cancer, while it is the inability of immune cells of the body to find and destroy the newly formed cancer cells when they are few in numbers. The risk of cancer is increased in those individuals, whose immune system is suppressed due to any factor including chronic stress, old age, chronic debilitating disease, previous use of chemotherapy, and abuse of drugs such as antibiotics, corticosteroids, and analgesics.

Cancer cells and normal cells are different on many levels. Some of the differences are well known, but others have just been recently discovered and are less well understood.

The most important differences between cancer cells and normal cells are (Figure32):

- Normal cells divide only when they receive a set of appropriate signals whereas cancer cells divide themselves despite the absence of those signals, and they are resistant to the signals telling them to self-destruct, known as apoptosis or programmed cell death.
- Cancer cells fuel their growth with different nutrients than normal cells and some utilize different pathway to transform those nutrients into energy, allowing them to proliferate faster than normal cells. For instance, tumor can induce new blood vessels formation (Angiogenesis), bringing in more nutrients supply.
- Normal cells stop dividing when touching other cells. They usually do not migrate to other areas. Cancer cells, on the contrary, invade into surrounding tissues and spread to other organs.
- Cancer cells can evade our immune system elements which normally eliminate abnormal or invading cells. They can co-opt our immune system to help them proliferate. For instance, cancer cells can disguise as normal cells via antigen expression on the cell membrane.
- Cancer cell genes can be amplified, deleted, or altered. Their chromosomes can be reshuffled.

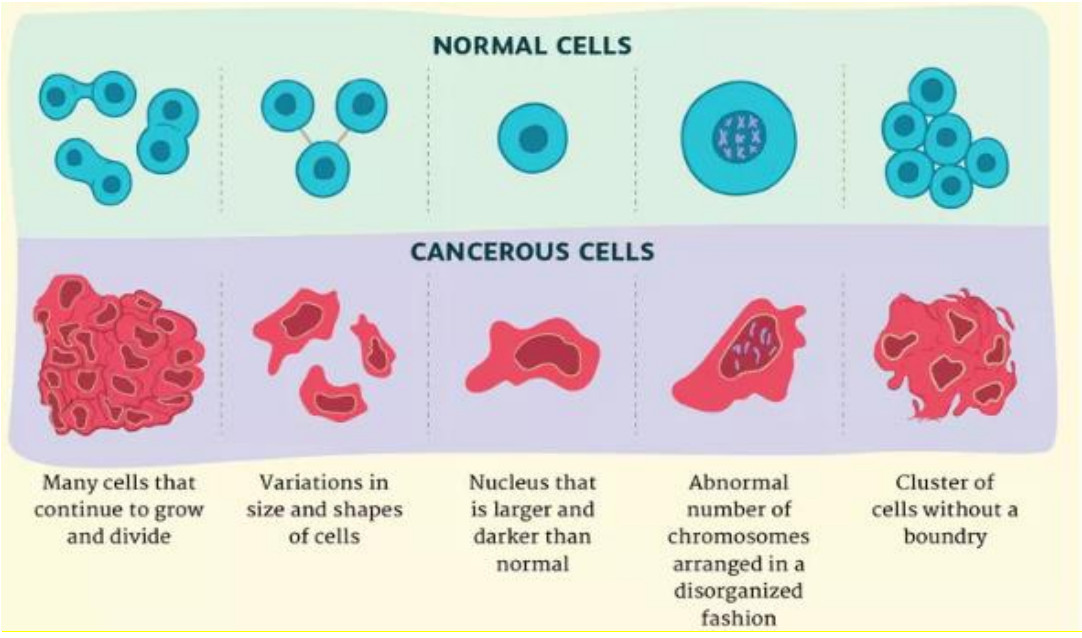

Figure 32: Differences between cancer and normal cells.

Table III: Basic differences between cancer and normal cells.

	Normal Cells	**Cancerous Cells**
Growth	Stop when there's enough	Uncontrolled growth
Communication	Respond to signals from other cells	Do not respond to signals from other cells
Cell repair/death	Aged/damaged cells are repaired or replaced	Cells are neither repaired or replaced
Stickiness/spread	Stay together in assigned area	Can travel solo and throughout the body
Appearance	Uniform look under a microscope	Varied sizes, larger and darker center under a microscope
Maturation	Reach maturity	Do not reach maturity
Evasion of immune system	Can be targeted and eliminated	Can "hide" and grow uninterruputed
Function	Perform designated tasks	Fail to perform designated tasks
Blood supply	Blood vessels grow to feed normal growth and aid in repairs	Blood vessels grow regardless, constantly "feeding" a tumor

7.6. Benign and malignant tumors

Cancer can result from abnormal proliferation of any of the different kinds of cells in the body, so there are more than a hundred distinct types of cancer, which can vary substantially in their behavior and response to treatment. The most important issue in cancer pathology is the distinction between benign and malignant tumors

7.6.1. Benign tumors

The tumor is concluded as benign, if the cells are non-cancerous. It won't invade nearby tissues or spread to other areas of the body (metastasize). Benign tumors can be removed by surgery. They usually don't reoccur once removed, but if they do it

is usually in the same place. The non-surgical procedure commonly followed is medication or by a measured quantity of radiation.

A benign tumor is less harmful unless it is present nearby any important organs, tissues, nerves, or blood vessels and causing damage. They can be dangerous, such as when they occur in the brain and crowd the normal structures in the enclosed space of the skull. They can press on vital organs or block channels. Also, some types of benign tumors such as intestinal polyps are considered as precancerous and are removed immediately to prevent them becoming malignant.

Symptoms of the growth of the tumor are related to the location where they are growing. The common types of benign tumors are adenomas, fibroids, hemangiomas, lipomas, meningiomas, myomas, nevi or moles, neuromas, osteochondromas, papilloma, etc. The common local symptoms of cancer may be due to the growth of the mass of the tumor or its process of ulceration. The growth of the mass in the brain may affect the normal functioning of the brain, the growth of a mass in the lungs can cause blocks in the bronchus, leading to various respiratory disorders. The growth of a mass in the breast tissues can lead to breast cancer. The ulceration of the mass leads to bleeding of the tumor and based on the location shows up various symptoms at various stages of the abnormalities. The growth of the masses with the ulcerations may be painless at times and the individual may feel the pain at the advanced stage of cancer. The systemic symptoms of the tumor occur due to the effects that are unrelated to direct or metastatic spread. The commonly observed symptoms are unexplained weight loss; fever, feeling fatigue, skin condition changes, etc..

7.6.2. Malignant tumors (Cancerous)

Malignant means that the tumor is made of cancer cells and it can invade nearby tissues. Some cancer cells can move into the bloodstream or lymph nodes, where they can spread to other tissues within the body, this is called metastasis. For example, breast cancer begins in the breast tissue and may spread to lymph nodes in the armpit if it’s not caught early enough and treated. Once breast cancer has spread to the lymph nodes, the cancer cells can travel to other areas of the body, like the bones or liver. The

breast cancer cells can then form tumors in those locations referred as secondary tumor.

7.7. Differences between benign and malignant tumors

There are many important differences between benign and malignant tumors which are as follows (Figure 33):

- **Growth rate:** Generally malignant tumors grow more rapidly than benign tumors.
- **Ability to invade locally:** Malignant tumors have the ability to invade the tissues around them.
- **Ability to spread at distance:** Malignant tumors may spread to other parts of the body using the bloodstream or the lymphatic system. Malignant tumors may also invade nearby tissues and send out fingers into them, while benign tumors don't. Benign tumors only grow in size at the place of their origin.
- **Recurrence:** Benign tumors can be removed completely by surgery as they have clearer boundaries, and as a result, they are less likely to reoccur. Malignant tumors may spread to other parts of body. They are more likely to reoccur.

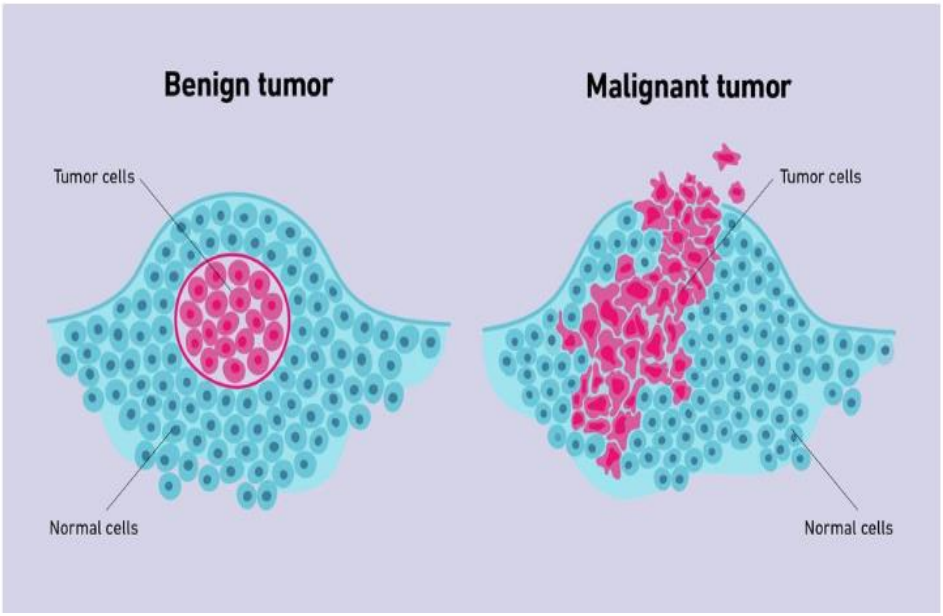

Figure 33: Differences between benign and malignant tumors.

7.8. DNA damage and repair pathways in cancers

7.8.1. DNA damage in cancer development

DNA is the biological template needed for an organism to develop, function, grow, and reproduce. Its integrity and stability are therefore vital to life. However, due to its dynamic nature, this macromolecule is constantly subjected to several alterations. In fact, it has been estimated that each cell of the human body receives approximately 70,000 DNA lesions per day. These aberrations arise from physiological or metabolic sources, as well as exogenous environmental influences.

Exposure to carcinogens is associated with various forms of DNA damage such as single-stand breaks, double-strand breaks, covalently bound chemical DNA adducts, oxidative-induced lesions and DNA–DNA or DNA–protein cross-links (Figure 34).

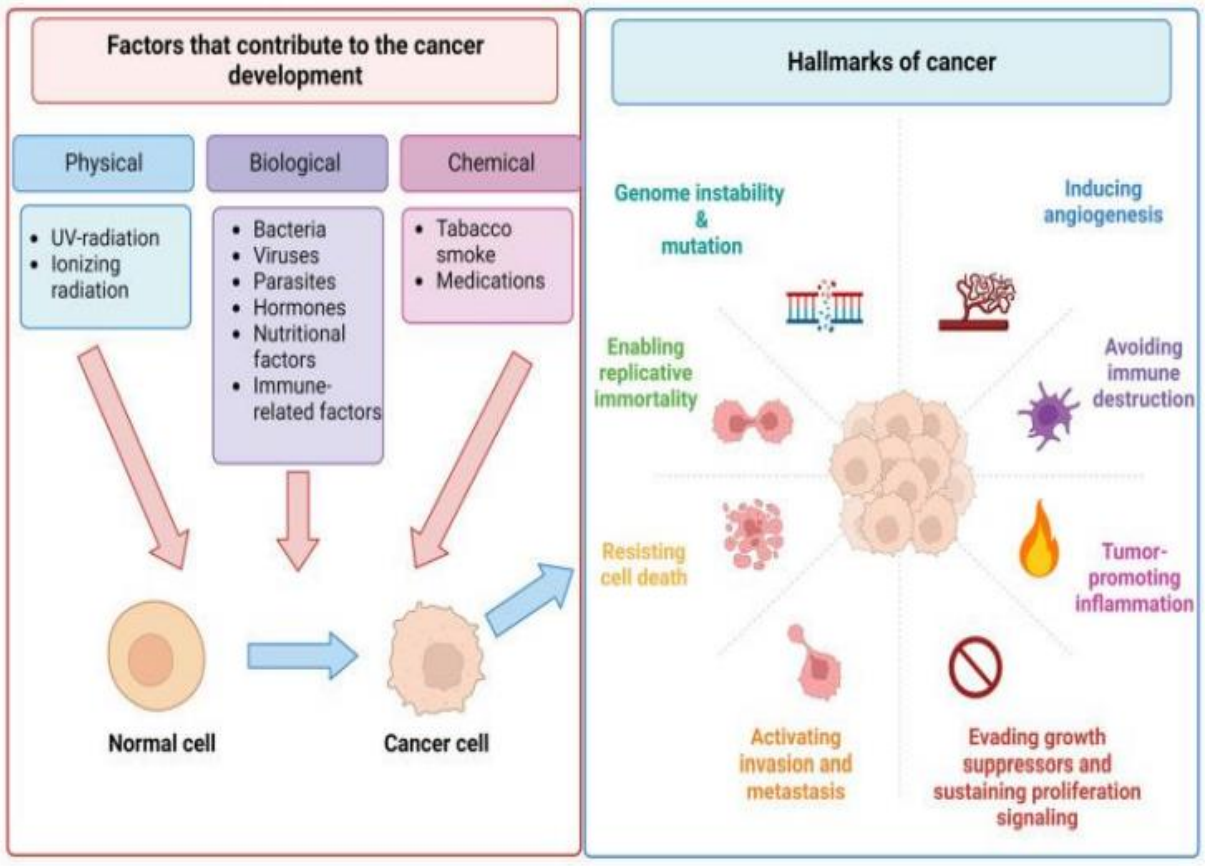

Figure 34: The main causes of human cancers and hallmarks of cancer established.

To avoid detrimental consequences to cellular functions and hence survival, life has evolved several systems that maintain genetic stability under strict control. Indeed, in addition to the proofreading activity of the DNA polymerases that correct mis-incorporated bases during replication, cells possess various DNA repair mechanisms to restore the damaged molecule. Unfortunately, not all DNA lesions are efficiently repaired in an error-free manner, leading to the acquisition and accumulation of many mutations which can ultimately contribute to several diseases, including cancer.

7.8.2. DNA Repair Pathways

DNA damage and defects in the repair genes are responsible for the accumulation of mutations and cancers. Cancer development or mutagenesis is highly related to impairment of DNA damage repair. Measuring the levels of DNA damage gives an overview of the level of carcinogenic chemicals leading to tumor genesis during the activation of repairing mechanisms after the damage occurs. Some factors such as the imbalance between DNA damage and repair also play a critical role in the accumulation of mutations in cancer cells. The frequency of mutation increases proportionally to the increase in the amount of DNA damage and reduction in the DNA repair.

The DNA damage response pathway is activated by cells in response to DNA damage. There are several types of cell responses such as cell-cycle arrest and stimulation of transcriptional and post-transcriptional mechanisms, which induce the genes associated with DNA repair and can activate programmed cell death in certain cases. Replication of genetic information and rearrangement are facilitated by the double-helical structure of DNA. Mostly, the effect of the DNA damage is harmful despite the fact that DNA mutation or recombination is the source of genetic variability and is essential for life after DNA damage. Several mechanisms can be activated to repair damaged DNA including direct repair, base excision repair (BER), nucleotide excision repair (NER), mismatch repair, DNA strand break repair, non-homologous end joining (NHEJ) and homologous recombination (HRR) (Figure 35) .

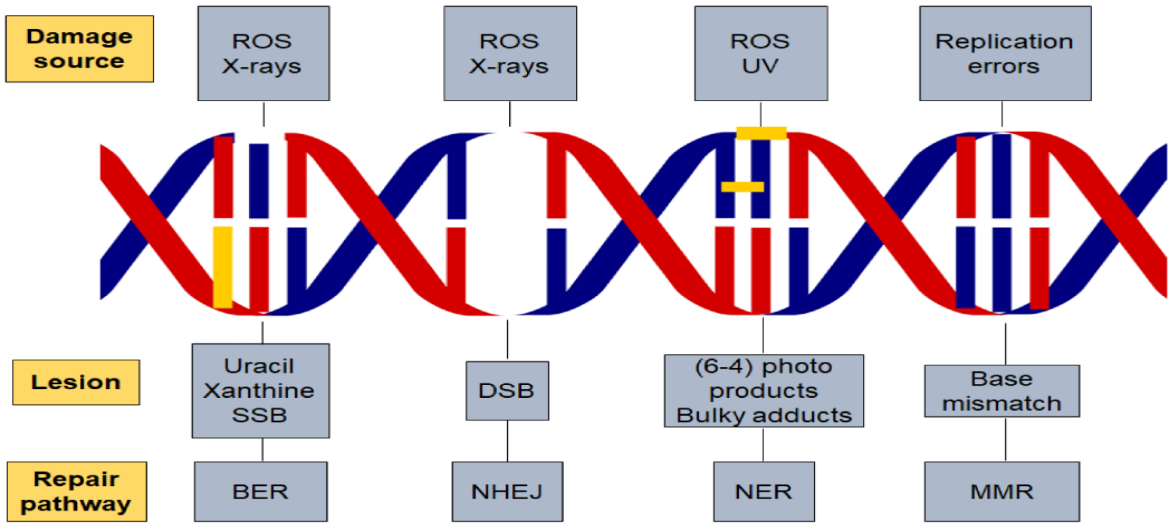

Figure 35: DNA damage and repair pathways. *Different factors are responsible for initiating DNA damage such as radiation and reactive oxygen species which cause several types of lesions in the DNA double helix. The repair pathway involved in the process is dependent on the damaging agent and lesion generated. Base excision repair (BER), nucleotide excision repair (NER), non-homologous end joining (NHEJ), reactive oxygen species (ROS) and DNA mismatch repair (MMR).*

7.9. Tumor microenvironment (TME)

Cancers represent complex ecosystems comprising tumor cells and a multitude of non-cancerous cells, embedded in an altered extracellular matrix. The tumor microenvironment (TME) includes diverse immune cell types, cancer-associated fibroblasts, endothelial cells, pericytes, and various additional tissue-resident cell types. These host cells were once considered bystanders of tumorigenesis but are now known to play critical roles in the pathogenesis of cancer. The cellular composition and functional state of the TME can differ extensively depending on the organ in which the tumor arises, the intrinsic features of cancer cells, the tumor stage, and patient characteristics. Here, we review the importance of the TME in each stage of cancer progression, from tumor initiation, progression, invasion, and intravasation to metastatic dissemination and outgrowth. Understanding the complex interplay between tumor cell-intrinsic, cell-extrinsic, and systemic mediators of disease progression is critical for the rational development of effective anti-cancer treatments (Figure 36).

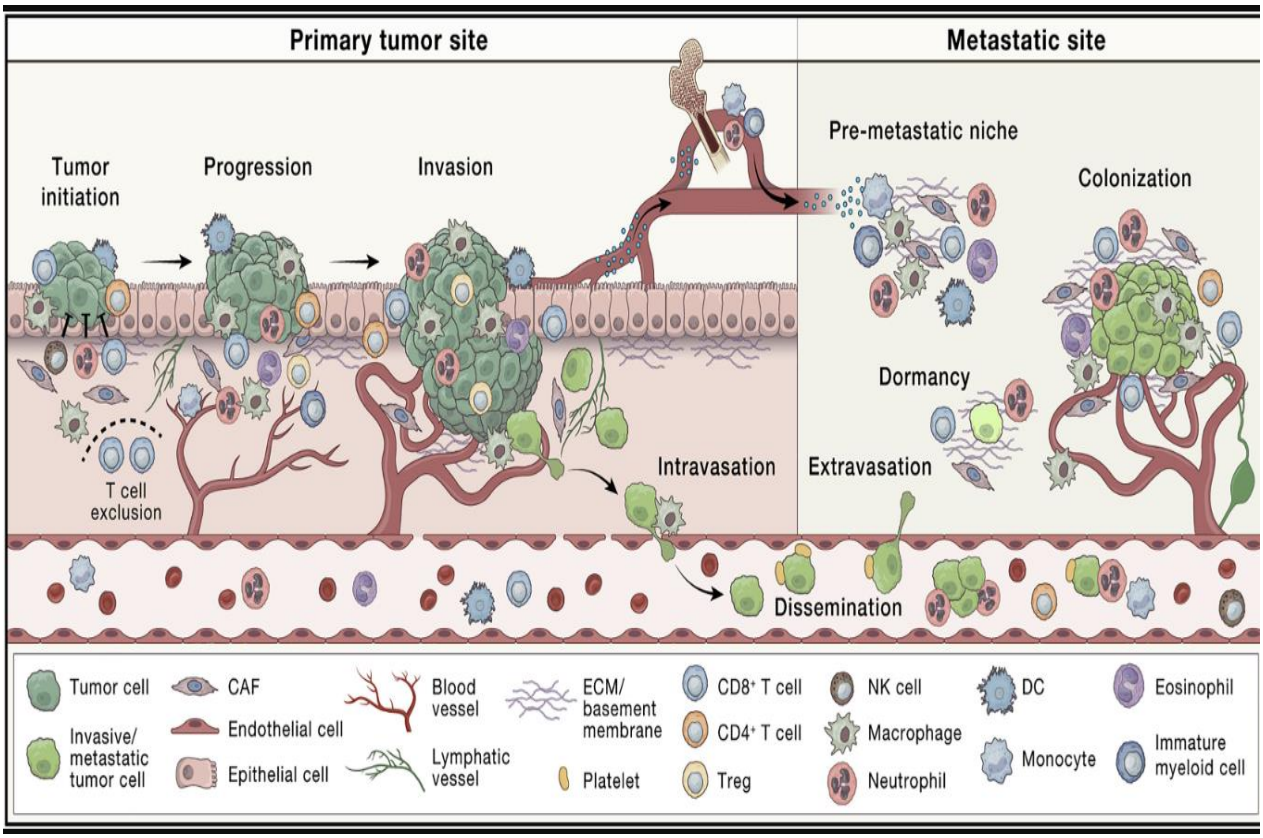

Figure 36: Microenvironmental regulation of primary tumor progression and metastasis. *The evolving tumor microenvironment (TME) during all stages of cancer progression is depicted with key representative cell types shown. The TME includes diverse immune cells, cancer-associated fibroblasts (CAFs), endothelial cells, and the extracellular matrix (ECM), among others. These components may vary by tissue type and co-evolve with the tumor as it progresses. The normal tissue microenvironment can constrain cancer outgrowth through the suppressive functions of immune cells, fibroblasts, and the ECM. However, for cancer to advance, it must evade these functions and instead influence cells in the TME to become tumor promoting, resulting in increased proliferation, invasion, and intravasation at the primary site. Cells and factors of the TME also play a vital role in preparing the premetastatic niche, regulating cancer cell survival in the circulation, and promoting extravasation. During the metastatic stages, the TME helps to control metastatic cell dormancy, emergence from this state, and subsequent metastatic outgrowth.*

Chapter II: Cell Organelles: Structure and Functions

Chapter II: Cell Organelles: Structure and Functions

1. Introduction

All organisms are composed of structural and functional units of life called 'cells'. The body of some organisms like bacteria, protozoans and some algae is made up of a single cell whereas the body of higher fungi, plants and animals are composed of many cells. Human body is built of about one trillion cells. Cells vary in size and structure as they are specialized to perform different functions. But the basic components of the cell are common to all biological cells.

The cellular components are called cell organelles. These cell organelles include both membrane and non-membrane bound organelles, present within the cells and are distinct in their structures and functions. They coordinate and function efficiently for the normal functioning of the cell. A few of them function by providing shape and support, whereas some are involved in the locomotion and reproduction of a cell.

2. Cell definition

A cell may be defined as a unit of protoplasm bound by a plasma or cell membrane and possessing a nucleus. Protoplasm is the life giving substance and includes the cytoplasm and the nucleus. The cytoplasm has in it organelles such as ribosomes, mitochondria, golgi bodies, plastids, lysosomes and endoplasmic reticulum. Plant cells have in their cytoplasm, large vacuoles containing non-living inclusions like crystals, and pigments. The bacteria have neither defined cell organelles nor a well formed nucleus. But every cell has three major components: plasma membrane, cytoplasm, DNA (naked in bacteria) and enclosed by a nuclear membrane in all other organisms (Figure 37).

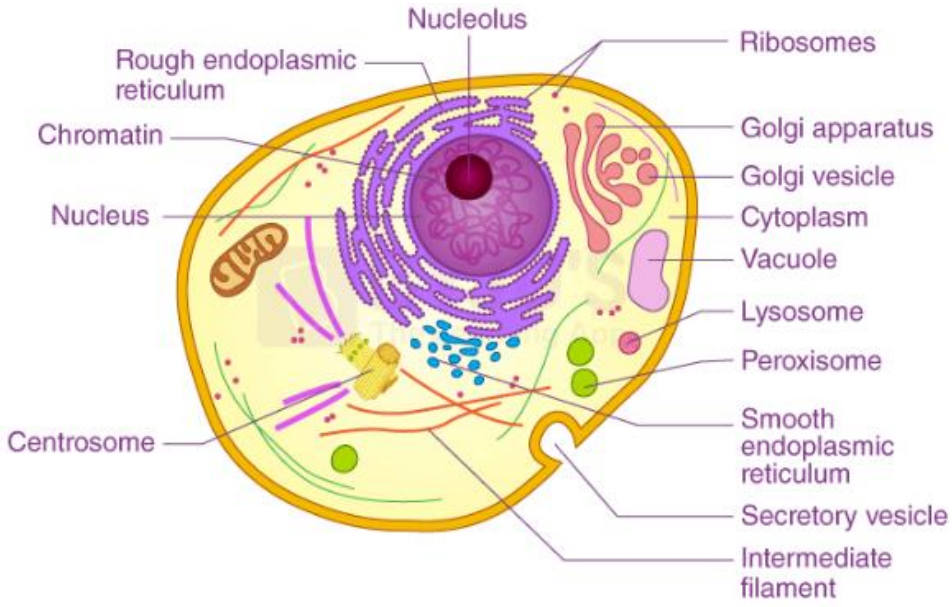

Figure 37: Animal cell structure.

3. Prokaryotic and eukaryotic cell

Cell is the structural and functional unit of life and it contains all necessary infrastructure to peform all functions. Based on the nuclear organisation and membrane-bound cell organelles, cells are classified as prokaryotic and eukaryotic cells. Some of the components are common to both prokaryotic and eukaryotic cell (Table IV). These are plasma membrane, cytoplasm, ribosomes, DNA, etc. Prokaryotic cells are without an organised nucleus and contain numerous ribosomes, mesosomes (folds in plasma membrane) besides having locomotory structures such as flagella in some of them. While a eukaryotic cell has a well-organised nucleus, cel lmembrane and membrane-bound cell organelles such as endoplasmic reticulum, golgi apparatus, mitochondria, plastids, vacuole, lysosomes, peroxisomes, and many more **(**Figure 38).

Table IV: Difference betweenn prokaryotic and eukaryotic cells.

	Prokaryotic cells	**Eukaryotic cell**
Examples	bacteria, archaea	protists, fungi, plants, animals (humans)
Nucleus	nucleoid region (no true nucleus)	nucleus with double membrane
Size	~ 1–5 μm	~ 10–100 μm
DNA	usually circular	linear chromosomes with histone proteins
RNA/protein synthesis	coupled in the cytoplasm	RNA synthesis in nucleus protein synthesis in cytoplasm
Ribosomes	50S and 30S	60S and 40S
Chromosomes	single chromosome	more than one chromosome
Cell division	binary fission	mitosis (budding or fission) meiosis
Membranes	cell membrane only	cell membrane and membrane-bound organelles
Organization	usually single cells	single cells, colonies, multicellular organisms
Movement	flagella (with flagellin)	flagella (with microtubules), cilia, lamellipodia, filopodia
Mitochondria	none	one to thousands
Chloroplasts	none	mainly in plants and algae
Internal structure	relatively sparse	complex

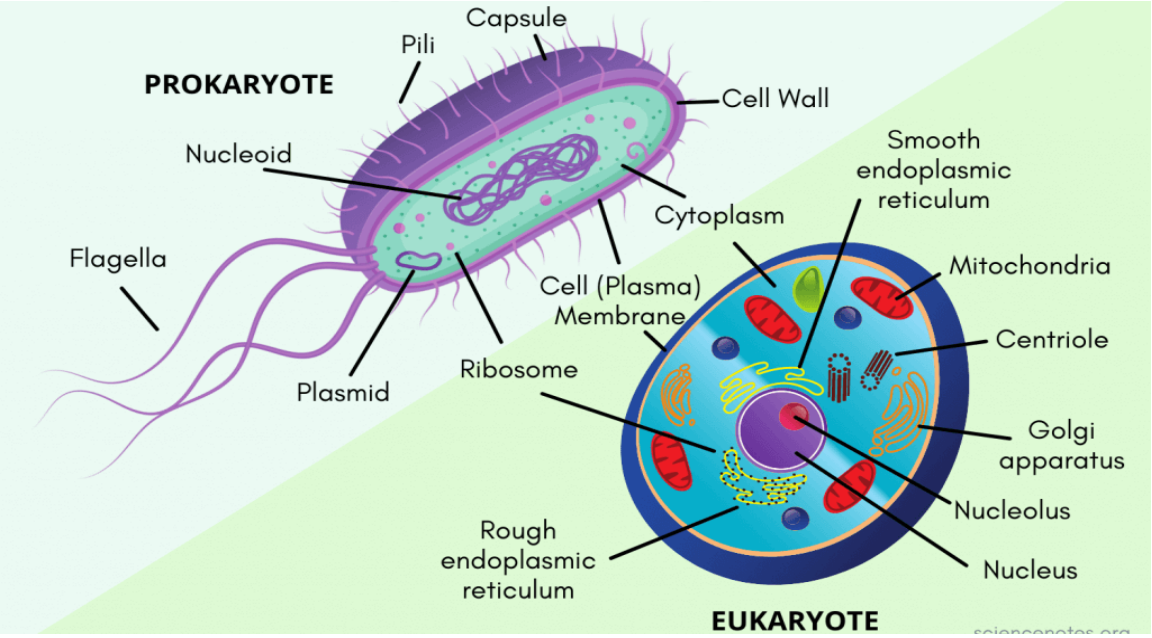

Figure 38: Difference between prokaryotic and eukaryotic cells.

4. Cell Organelles and their Functions

Organelles are the main structural and functional subunits within our cells. They are the cellular factories and powerhouses involved in maintaining cell health and homeostasis. Disruption of specific organelle functions can be a cause or a symptom of cancer, neurodegeneration, and rare genetic diseases, among others. These organelles are membrane-bound structures that each play a unique role and contain a specific panel of proteins and other molecules. The description of different structural feature of eukaryotic cell is as follows:

4.1. Cell membrane

The cell membrane is also termed as a plasma membrane or cytoplasmic membrane. The cell membrane is a living boundary that separates the living contents of the cell from the nonliving surrounding environment. Inside the cell, the nucleus is surrounded by the cytoplasm, a semifluid medium that contains organelles. The plasma membrane regulates the entrance and exit of molecules into and out of the cytoplasm.

- **Structure**

  The cell membrane is a selectively permeable membrane of the cells, which is composed of a phospholipid bilayer and proteins. Phospholipid molecules have a hydrophilic head and a hydrophobic tail. Both layers have the head on the outside and the tail on the inside. Such a structure makes it difficult for macromolecular substances and polar molecules to enter and exit. But there are also some channels on the cell membrane that are controlled by the membrane potential, allowing the corresponding molecules to enter.

  Two different types of proteins have been identified in the plasma membrane based on their location and association; peripheral and integral membrane proteins. Peripheral membrane proteins are mainly involved in cell signalling and these are superficially attached to lipid bilayer. Integral membrane proteins are partially or fully buried in the plasma membrane. Transmembrane proteins are the most abundant type of integral membrane protein (Figure 39).

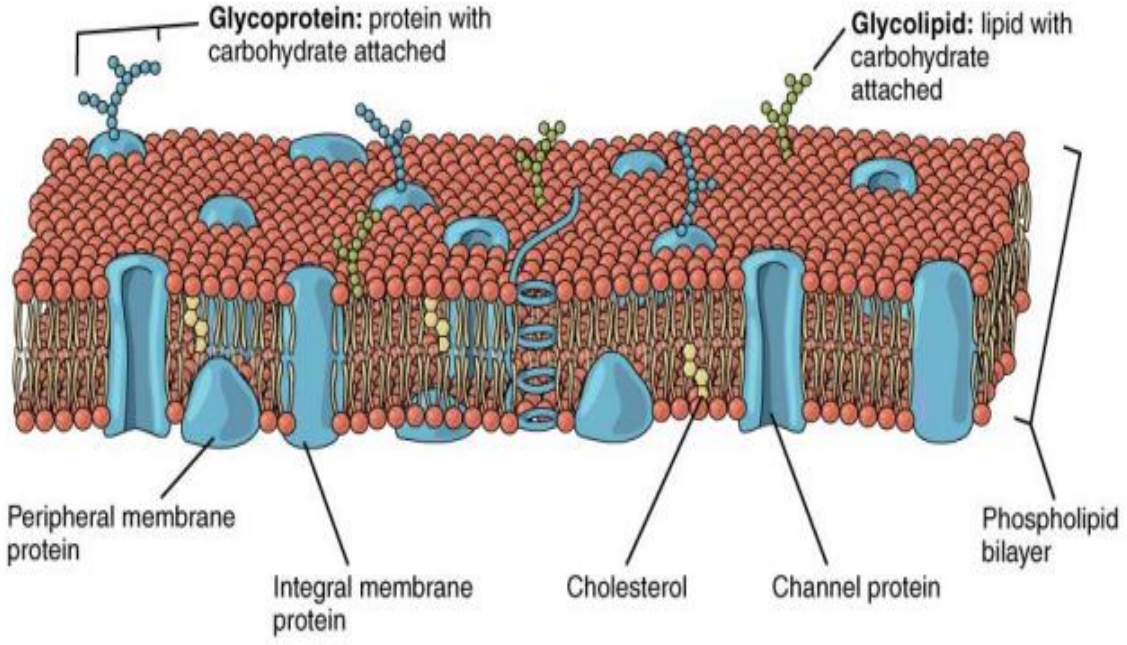

Figure 39: Cell membrane.

The cell membrane is a phospholipide bilayer containing different molecular compounds, including proteines and cohlesterol, some with carbohydrate groups attached.

- **Function**

  This membrane is composed of a phospholipid bilayer implanted with proteins. It forms a stable barrier between two aqueous compartments, which are towards the outside and inside of a cell in plasma membrane.

  The **lipid bilayer** serves as a barrier keeping proteins, ions and various other molecules where it is required and prevent its inaccurate diffusion. These are impermeable to most of the hydrophilic molecules. In particular, bilayers are impermeable to ions that allow cells to regulate pH and salt concentrations by transportation of ions across its membrane with the use of ion pumps. The **embedded proteins** function as either channels or transporters of compounds across the membrane, as receptors for the binding of hormones and neurotransmitters, or as structural proteins. The **peripheral membrane proteins** provide mechanical support to the membrane through the inner membrane skeleton or the cortical skeleton.

  Plasma membrane renders protection to the cell along with providing a fixed environment within the cell. It is responsible for performing different functions. It also maintains the cell potential. Plasma membrane is responsible for interacting with other adjacent cells, which can be glycoprotein or lipid proteins.

  The cell membrane plays an extremely important physiological function in the cell structure. Not only does it regulate and select the movement of substances into and out of the cell, but the cell maintains a stable internal environment.

4.2. Cytoplasm

Cellular cytoplasm is the context for all intracellular activities that are not sequestered within membrane-bounded organelles, and thus its physical chemical properties influence key cellular functions, including protein folding, enzyme catalysis, intracellular signaling, intracellular transport, and localization of molecules and organelles, as well as the fate of nanoparticles and therapeutic agents targeted to cells.

- **Structure**

  The cytoplasm is mainly composed of water, ions, organic molecules (such as proteins, carbohydrates, and lipids), and organelles (such as mitochondria, endoplasmic reticulum, Golgi apparatus, etc.). This intracellular jelly-like substance provides support for the cell's internal structure, allows the cell to maintain its shape, and aids in the transport of substances. The water in the cytoplasm acts as a solvent, enabling chemical reactions to take place within it.

- **Functions**

  The cytoplasm is the site of many biochemical reactions. Many enzymes and metabolic pathways within cells require the cytoplasm to occur. In addition, the cytoplasm is also the site of intracellular molecular transport. This includes the transport of substances via vesicles that can move through the cytoplasm, delivering substances from one organelle to another. The cytoplasm also plays a role in supporting the organelles so that they can function properly. However, when abnormalities occur in the cytoplasm, serious diseases can result. For example, certain genetic diseases are associated with defects in specific proteins within the cytoplasm. The cytoplasm can also be affected by viral infection. Certain viruses require access to the cytoplasm in order to replicate and spread. They may promote their own growth by altering the cytoplasmic environment, leading to cellular dysfunction and ultimately disease.

4.3. Mitochondria

Mitochondria (singular: mitochondrion) are found in nearly all eukaryotic cells. Some cells may have single large mitochondrion, but more often, a cell has hundreds or even thousands of mitochondria at variable location in cells depending upon the cell function.

- **Structure**

  Mitochondria are around 0.5−1.0 micrometres (μm) in diameter. Each mitochondrion is a double membrane bound structure; outer and inner membrane, each consisting of phospholipids bilayer with proteins (Figure 40).

  Between these membranes is the intra-membrane space. The inner membrane encloses a plasmatic mitochondrial matrix. The outer membrane is smooth, but

the inner membrane has infoldings called cristae (singular: crista) that provides it comparatively larger surface area.It consists of approximately 70% protein and 30% lipid. Most of these proteins are directly involved in respiration.

The inner membrane and matrix contains all enzymes and proteins involved in the process of tricarboxylic acid (TCA) cycle and cellular respiration for the purpose of ATP synthesis. Mitochondria also contains DNA molecules, ribosome (70S) and few RNA molecules. Some of the mitochondrial proteins are synthesised by genes present on the mitochondrial DNA. Therefore, mitochondrial matrix is the site of organellar DNA replication, transcription, protein synthesis and other enzymatic processes.

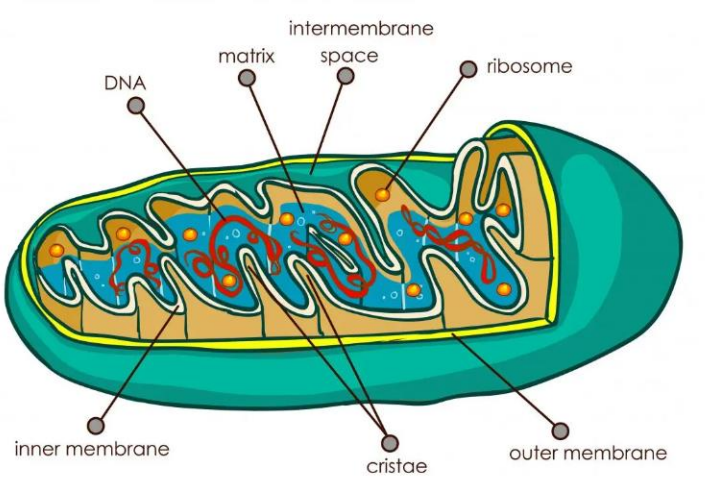

Figure 40: Structure of mitochondria. *The mitochondrion is composed of a double membrane: the inner membrane and the outer membrane. Between these membranes is the intra-membrane space. The inner membrane has infoldings called cristae The mitochondrial matrix contains several copies of mitochondrial circular DNA and ribosomes.*

- **Function**

Mitochondria, often called "powerhouses" of the cell, produce adenosine triphosphate (ATP), the energy currency of most cellular processes. All membrane proteins that are involved in the oxidative phosphorylation are located at the inner membrane. These proteins pump protons into the intermembrane space driven by an electron transfer. The proton gradient is used

by the membrane enzyme ATP synthase to form ATP. Highly convoluted cristae structures of the inner membrane increase surface area to maximize ATP yield through the electron transport chain**.**

The outer membrane has an enzyme composition that is very different from that of the inner membrane. It binds mainly membrane pore proteins, transports, and enzymes that are involved in amino acid oxidation, phospholipid synthesis, and other reactions**.**

The matrix accommodates the enzymes of the Krebs Cycle and fatty acid degradation (β-oxidation) and a whole machinery for protein and nucleic acid synthesis.

Additionally, mitochondria have important roles in regulating apoptosis, cellular redox homeostasis, intracellular calcium signaling, and neurogenesis

4.4. Cell nucleus

The nucleus is a characteristic feature of most eukaryotic cells. It is considered to be one of the most important structures of eukaryotic cells as it serves the function of information storage, retrieval and duplication of genetic information. It is a double membrane-bound organelle that houses the genetic material in the form of chromatin. It consists of a dynamic mix of non-membranous subcompartments of varying functional capacity. The specific features of a cell nucleus, especially in terms of the nature and distribution of the subnuclear compartments and the positioning of the chromosomes, depend upon their differentiated state in the organism.

- **Structure**

  In mammalian cells, the average diameter of the nucleus is approximately 6 mm, which occupies about 10% of the total cell volume. The nucleus is a complex and highly compartmentalized organelle, which undergoes major organization changes during cell differentiation, allowing cells to become specialized and fulfill their functions.

  The cell nucleus is a complex organelle, in which the genetic material (DNA) is compacted up to several thousand fold and organized into chromosomes in a

form that allows the genome to be replicated, repaired, and transcribed in a cell type–specific manner (Figure 41).

The cell nucleus consists of a nuclear membrane, called the nuclear envelope, nucleoplasm, nucleolus, and chromosomes. Nucleoplasm, also called karyoplasm, is the matrix present inside the nucleus. The nuclear membrane separates the constituents of the nucleus from the cytoplasm. Like the cell membrane, the nuclear envelope consists of phospholipids that form a lipid bilayer. The nuclear membrane is continuous with the endoplasmic reticulum. The envelope helps to maintain the shape of the nucleus and assists in coordinating the flow of the molecules into and out of the nucleus through nuclear pores. The nucleus of the cell contains DNA.

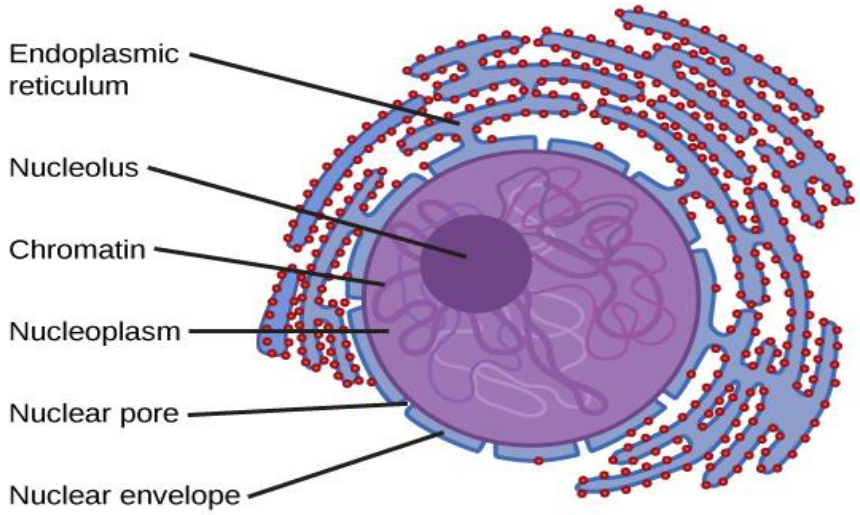

Figure 41: Cell nucleus. *The nucleus stores chromatin (DNA plus proteins) in a gel-like substance called the nucleoplasm. The nucleolus is a condensed region of chromatin where ribosome synthesis occurs. The boundary of the nucleus is called the nuclear envelope. It consists of two phospholipid bilayers: an outer membrane and an inner membrane. The nuclear membrane is continuous with the endoplasmic reticulum. Nuclear pores allow substances to enter and exit the nucleus.*

- **Function**

The key functions of the cell nucleus include deoxyribonucleic acid replication and further to control gene expression during the cell cycle. Transcription and further post-transcriptional processing of pre-messenger ribonucleic acids

(mRNAs) occur inside the nucleus and the mature mRNAs are transported into the cytoplasm where the translational events occur. Thus, nucleus provides functional compartmentalisation inside the cell allowing higher levels of gene regulation. The cell nucleus controls the hereditary characteristics of an organism.

4.5. Endoplasmic reticulum

The hallmark of eukaryotic cells is the packing of DNA into the nucleus, a compartment that is bounded by the nuclear envelope. The nuclear envelope is continuous with the endoplasmic reticulum, which suggests that the endoplasmic reticulum was present in eukaryotic cells right from their 'inception.'

The endoplasmic reticulum (ER) is a large, dynamic structure that serves many roles in the cell including calcium storage, protein synthesis and lipid metabolism. The diverse functions of the endoplasmic reticulum are performed by distinct domains; consisting of tubules, sheets and the nuclear envelope. Several proteins that contribute to the overall architecture and dynamics of the endoplasmic reticulum have been identified, but many questions remain as to how the endoplasmic reticulum changes shape in response to cellular cues, cell type, cell cycle state and during development of the organism.

Work from a variety of systems has revealed that the endoplasmic reticulum is composed of multiple different structural domains, each of which is associated with a specific function or functions. However, it is not yet clear how these functional subdomains are organized and how different functional domains translate into different structures.

- **Structure**

  The endoplasmic reticulum (ER) is a large membrane-bound compartment spread throughout the cytoplasm of eukaryotic cells. It is a network of membranous tubules and sacs that extend throughout the cytoplasm of eukaryotic cells. It consists of two distinct regions: the rough endoplasmic reticulum (RER) and the smooth endoplasmic reticulum (SER).

  Rough endoplasmic reticulum is studded with ribosomes on its cytoplasmic surface, giving it a "rough" appearance when viewed under a microscope.

Unlike the rough endoplasmic reticulum, the smooth endoplasmic reticulum (SER) lacks ribosomes on its surface.

The endoplasmic reticulum is divided into three major morphologies that include the nuclear envelope, sheet-like cisternae, and a polygonal array of tubules connected by three-way junctions (Figure 42). The relative amounts of these different domains vary greatly depending on the cell type. Invariably, however, they are all part of a single interconnected membrane system that contains a common luminal space and often extends to the farthest reaches of the cell. ER sheets are relatively flat areas where the membrane extends for many microns with little membrane curvature. Although the nuclear envelope is spherical, the nucleus is so large that its surface can also be considered as a flat ER sheet. In contrast, ER tubules are long cylindrical units with high membrane curvature in cross-section in higher eukaryotes, the peripheral ER extends throughout the entire volume of the cytoplasm. Regardless of the cell type or subcellular location, the thickness of a sheet and the diameter of a tubule is typically 60–100 nm.

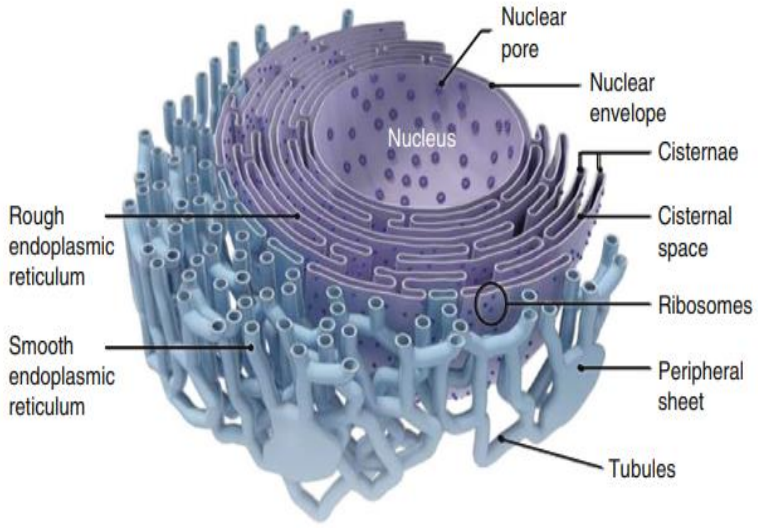

Figure 42: Schematic of the endoplasmic reticulum in animal cell.

The nucleus is surrounded by a system of two concentric membranes, called the inner and outer nuclear membranes. The outer nuclear membrane is continuous with the endoplasmic reticulum, so the space between the inner and outer nuclear membranes is directly connected with the lumen of the endoplasmic reticulum.

- **Function**

The endoplasmic reticulum is a versatile organelle with a wide range of functions that are vital for cellular homeostasis and proper functioning. Some of its key roles include:

Protein Synthesis and Folding; The RER's ribosomes synthesize proteins that are destined for secretion, incorporation into the cell membrane, or localization within lysosomes. These proteins often undergo intricate folding processes within the RER's lumen, assisted by chaperone proteins. Proper folding is crucial for the protein's functionality.

The SER is involved in lipid metabolism, including the synthesis of phospholipids and steroids. The SER contains enzymes that aid in detoxifying drugs and various toxins, making them more water-soluble and easier to eliminate from the body. This function is especially important in liver cells, which are responsible for processing and detoxifying a wide range of substances.

In certain cell types, such as muscle cells, the SER plays a role in regulating calcium levels, which is crucial for muscle contraction. A wealth of signaling pathways exploits the $Ca2^{+}$ gradient across the ER membrane. Typically, signaling cascades drive the opening of $Ca2^{+}$ channels to sustain a burst of $Ca2^{+}$through vicinal mitochondria and in the cytosol, which, in turn, serves to propagate the signal. In most cases, there is a rapid reuptake of $Ca2^{+}$ back into the ER, ensuring that $Ca2^{+}$signaling can start anew. The most sophisticated $Ca2^{+}$ driven signaling cycles occur at the sarcoplasmic reticulum to sustain muscle contraction

Glycosylation is essential to the synthesis, folding, and function of glycoproteins in eukaryotes. Proteins are co- and posttranslationally modified by a variety of glycans in the endoplasmic reticulum (ER); modifications include C- and O-mannosylation, N-glycosylation, and the addition of glycosylphosphatidylinositol membrane anchors. Protein glycosylation in the ER of eukaryotes involves enzymatic steps on both the cytosolic and lumenal surfaces of the ER membrane.

Both regions of the ER contribute to the synthesis of new cell membrane components. The rough ER produces membrane proteins and phospholipids, while the smooth ER is involved in synthesizing lipids used in membranes.

The endoplasmic reticulum is not an isolated organelle, it interacts closely with other cellular components. For example, proteins synthesized in the RER may travel to the Golgi apparatus for further processing and sorting before reaching their final destinations. Additionally, the ER is connected to the nuclear envelope, allowing for the exchange of molecules between the ER and the nucleus.

4.6. Golgi apparatus

The eukaryotic cells have various membraneous and nonmembranous organelles within their cytoplasm. According to the scientists, the Golgi apparatus is commonly considered as the most important organelle.

Golgi apparatus or golgi complex, or golgi body, or the Golgi, is found in all plant and animal cells. The Golgi complex is a central organelle in the endomembrane system. Although it has been detected many years ago, several unexpected morphological or functional features of the Golgi apparatus are still emerging.

Elaboration of a detailed model of Golgi apparatus functioning has proved difficult because of the extremely high diversity of the organelle, including its morphology, position in the cell, intensity of its activity, and the nature of its products.

- **Structure**

  Golgi apparatuses extremely dynamic and pleomorphic structure because of its variable shape and form in different cell types. In mammalian cells the Golgi apparatus consists of a series of flattened cisternal stacks located in the perinuclear region of the cell. Several conditions cause the lace-like ribbon structure to undergo profound morphological changes.

  The Golgi apparatus consists of compact stacks of membrane limited sacs or cisternae, dilated at the periphery, along with many vesicles involved in vesicular transport between the sacs (Figure 43). In mammalian tissue culture cells, it consists of flattened membrane-bound compartments, called “cisternae”. The cisternae form Golgi stacks (one Golgi stack is formed via

grouping of a number of cisternae in a parallel manner), themselves interconnected by lateral tubules to form the Golgi ribbon. The Golgi ribbon displays a juxtanuclear localisation next to the microtubule organising center. The Golgi ribbon is intact in interphase and dispersed into tubularreticular and vesicular elements in mitosis. Additionally, in the absence of intra-Golgi transport, the Golgi ribbon is partially fragmented. Both Golgi stacks and ribbon are polarised with an entry (cis) face, where cargo molecules synthesised in the endoplasmic reticulum (ER) reach the Golgi, and an exit (trans) face, where they leave for their downstream locations.

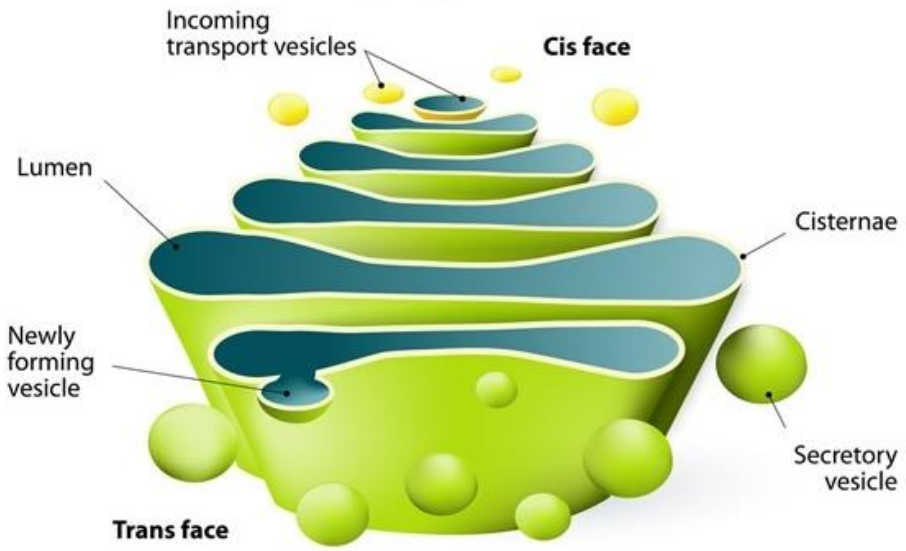

Figure 43: Golgi apparatus. *The Golgi apparatus is made up of approximately four to eight cisternae, although in some single-celled organisms it may consist of as many as 60 cisternae. The three primary compartments of the apparatus are known generally as "cis" (cisternae nearest the endoplasmic reticulum), "medial" (central layers of cisternae), and "trans" (cisternae farthest from the endoplasmic reticulum).*

- **Function**

  The primary function of the Golgi is to modify and package proteins and lipids into transport carriers and send them to the proper locations. Secretory or transmembrane proteins are delivered from the endoplasmic reticulum (ER) to the cis-Golgi network. Subsequently, the cargo molecules travel through the different cisternae of the Golgi where they are posttranslationally modified by resident enzymes. Modifications include glycosylation, phosphorylation,

sulfation, and proteolysis. Cargo molecules have been described to move through the Golgi stack in several different ways. One possible mechanism is through cisternal maturation, in which the cargo remains in the cisternae and new cisternae form at the cis side. The newly formed cisternae then mature by receiving Golgi enzymes via retrograde transport of COPI vesicles. Alternatively, proteins are shuttled by COPI vesicles between cisternae, whereas Golgi resident enzymes reside in the relatively stable cisternal compartments. In addition, cargo molecules may flow through transient tubular connections between adjacent cisternae, or by rapid partitioning between different lipid domains. At the trans-Golgi network (TGN), cargo molecules are sorted and transported to their proper destinations such as the endosomal-lysosomal system, the plasma membrane, or outside of the cell (Figure 44).

In addition to its function in protein trafficking, the Golgi in mammalian cells plays an important role in pathways controlling mitotic entry, cytoskeleton organization and dynamics, calcium homoeostasis, and PM (plasma membrane)- receptor-initiated and organelle-autochthonous signalling events that respond to growth signals and energy status.

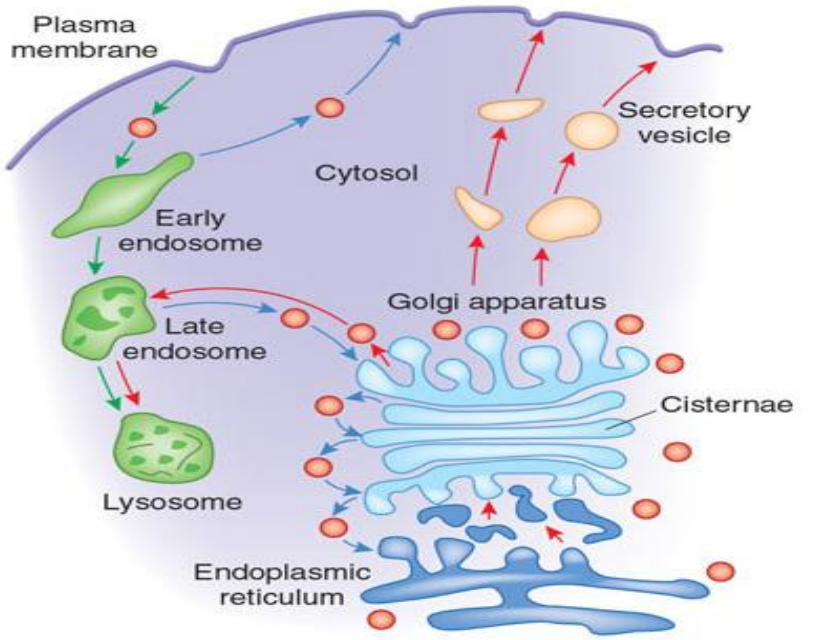

Figure 44 : The Golgi apparatus modifies and sorts proteins for transport throughout the cell. *The Golgi apparatus is often found in close proximity to the ER in cells. Protein cargo moves from the ER to the Golgi, is modified within the Golgi, and is then sent to various destinations in the cell, including the lysosomes and the cell surface.*

4.7. Lysosomes

Lysosomes are dynamic organelles that receive and degrade macromolecules from the secretory, endocytic, autophagic and phagocytic membrane-trafficking pathways. Lysosomes are membranous sacs filled with numerous hydrolysis enzymes. Cytological research has shown that lysosomes are formed by budding from the Golgi apparatus. Lysosomes degrade intracellular organelles that have reached the end of their life, as well as molecules taken from the extracellular environment by endocytosis.

- **Structure**

  Lysosomes are membrane-bound organelles that are present in animal cells. They can be distinguished from endosomes by the lack of mannose-6-phosphate receptors (MPRs).

  Lysosomes are small vesicles which are bounded by a single membrane and contain hydrolytic enzymes (acid hydrolases) in the form of minute crystalline or semi crystalline granules of 5-8 nm.

  Electron microscopy, which was not used in their initial discovery, subsequently showed that lysosomes constitute up to 5% of the intracellular volume and are of heterogeneous size and morphology; they often contain electron-dense deposits and membrane whorls (Figure 45).

  Lysosomes were shown to have proton-pumping vacuolar ATPases, which maintain the lumenal environment at a pH of 4.6–5.0

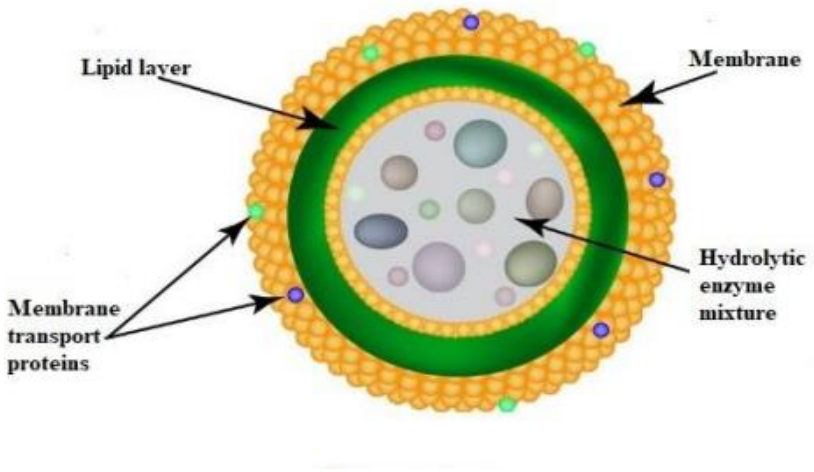

Figure 45: Lysosome structure.

- **Function**

  Not all the proteins produced and assembled by the ER and Golgi apparatus are destined to be transferred to the cell surface. In animal cells, independent subsets of proteins are sent to the lumens of other organelles, the lysosomes. Lysosomes are specialized vesicles containing enzymes involved in the breakdown of macromolecules such as sugars, proteins and fats. Although they can adopt a variety of irregular shapes, they are all characterized by an acidic lumen with a pH of around 5. This acidity of the surrounding environment is a preferential condition for the functioning of lysosomal enzymes.

  Various macromolecules destined to be broken down are transferred to the lysosomes via the vesicles. The breakdown products, such as amino acids and lipids, are then transported across the lysosomal membrane into the cytosol to be made available to the cell.

5. Transport of proteins between different cellular organelles

5.1. Introduction

Eukaryotic cells are highly compartmentalized into distinct membrane-bound organelles, a feature essential for fundamental as well as specialized cellular processes. To function, each membrane-bound organelle has a unique composition of proteins and lipids. Therefore, it is not surprising that highly specific transport mechanisms are required to direct molecules to defined locations and to ensure that the identity, and hence function, of individual compartments are maintained. Proteins contain structural information that targets them to their correct destination and many targeting signals have now been defined.

Intracellular protein transport is divided into two major pathways with opposite directions. The exocytosis pathway is for the secretion of cellular components, while the endocytosis pathway is the entry pathway of molecules from outside the cell.

5.2. Exocytosis and Endocytosis

Exocytosis and Endocytosis are the two important functions performed by the cell membrane. Exocytosis concerned with the moving out of material from within a cell and endocytosis is the movement of material just in opposite direction; from outside of a cell to inside (Figure 46).

Endocytosis and exocytosis are two mechanisms involved in the transport of matter through the lipid bilayer. Both endocytosis and exocytosis occur through the formation of vesicles. Eukaryotic cells ingest particles and macromolecules into the cell by endocytosis. Phagocytosis and pinocytosis are the major mechanisms, involved in endocytosis. Phagocytosis of pathogens leads to the defense of the host. The content in Golgi apparatus is secreted to the extracellular environment by exocytosis. The toxic material and other unwanted things are eliminated from the cell by exocytosis as well. The main difference between endocytosis and exocytosis is that endocytosis refers to taking in matter to the cell from external environment whereas exocytosis refers to exporting material out of the Golgi complex via secretory vesicles into the external environment (Figure 47).

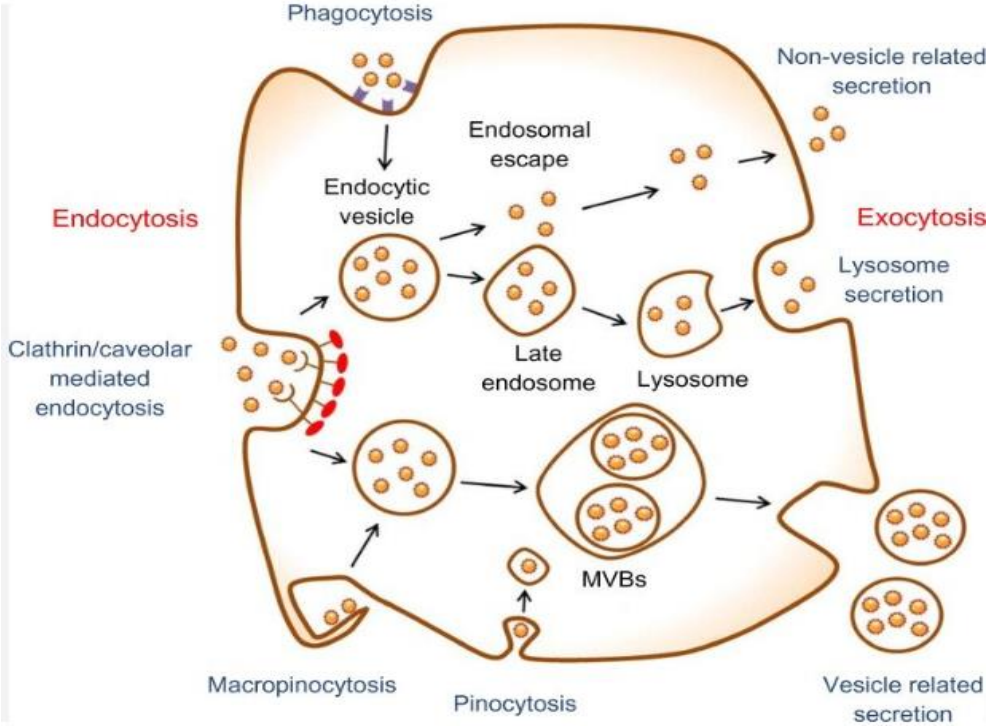

Figure 46: Illustration of the two types of vesicle transport, exocytosis and endocytosis.

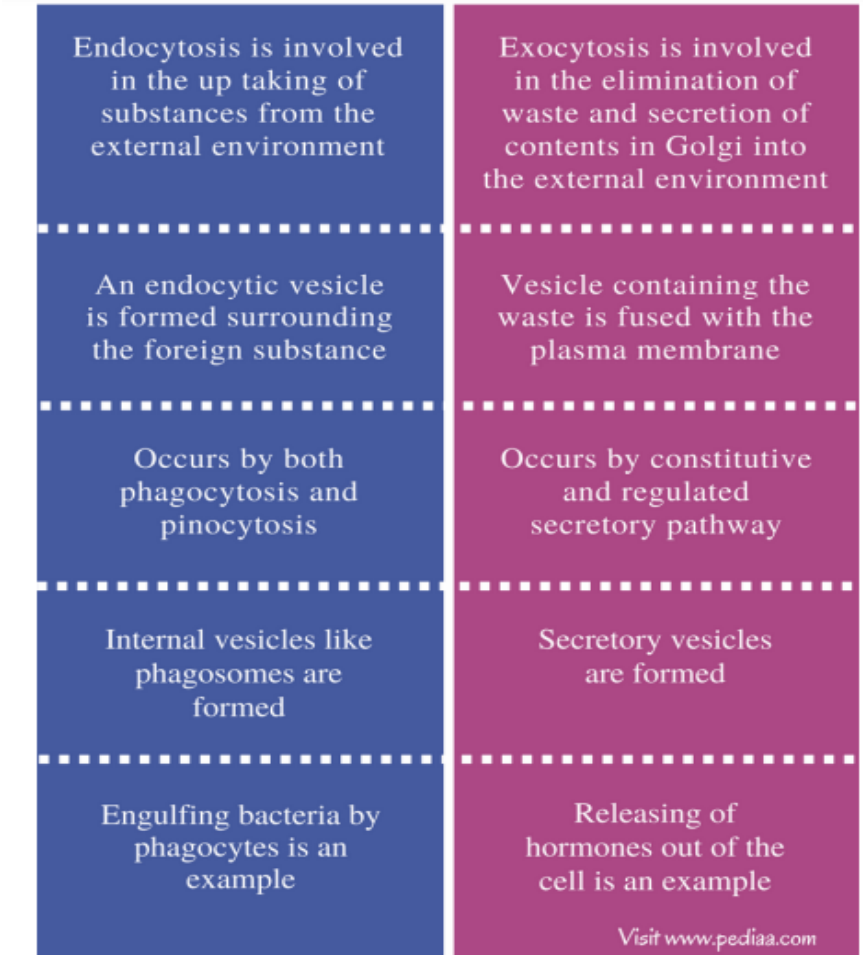

Figure 47: Main Difference between endocytosis and exocytosis.

5.2.1. Exocytosis and/or secretion pathways

Cells must ensure that each newly synthesized protein is correctly directed to its destination where it can perform an appropriate function. This process is called protein orientation or addressing; a protein can be intended to remain in the cytosol (e.g. glycolysis enzyme), it can also be oriented to an organelle (e.g. mitochondria, nucleus, etc.), be inserted into the plasma membrane or be exported outside the cell.

- If the protein is intended to remain in the cytosol, it is synthesized on free ribosomes and released directly into the cytosol.
- If the protein is intended for other locations, orientation mechanisms are involved. The notion of sorting signals is one of the fundamental concepts related to intracellular traffic.

All proteins originate on the ribosomes of the cytosol and, from there, are directed to 2 main branches (Figure 48):

- In the first branch, proteins are initially released into the cytosol after their synthesis. The majority of these proteins will remain in the cytosol, while others are exported to the mitochondria, the nucleus or the peroxisomes. The passage of proteins through the membrane of the mitochondria or peroxisomes is done by a translocation membrane protein. The passage of proteins into the nucleus is done through nuclear pores that have selective permeability.
- In the second branch, proteins are initially transferred to the endoplasmic reticulum. The transfer of these proteins to the endoplasmic reticulum occurs after fixation of the ribosomes that synthesize these proteins on the endoplasmic reticulum. Translocation requires a transport protein. Translocated proteins can remain in the endoplasmic reticulum, or they can be directed to the Golgi apparatus. In the Golgi apparatus, proteins can again remain in this compartment, or they can be directed either to lysosomes or to the plasma membrane.

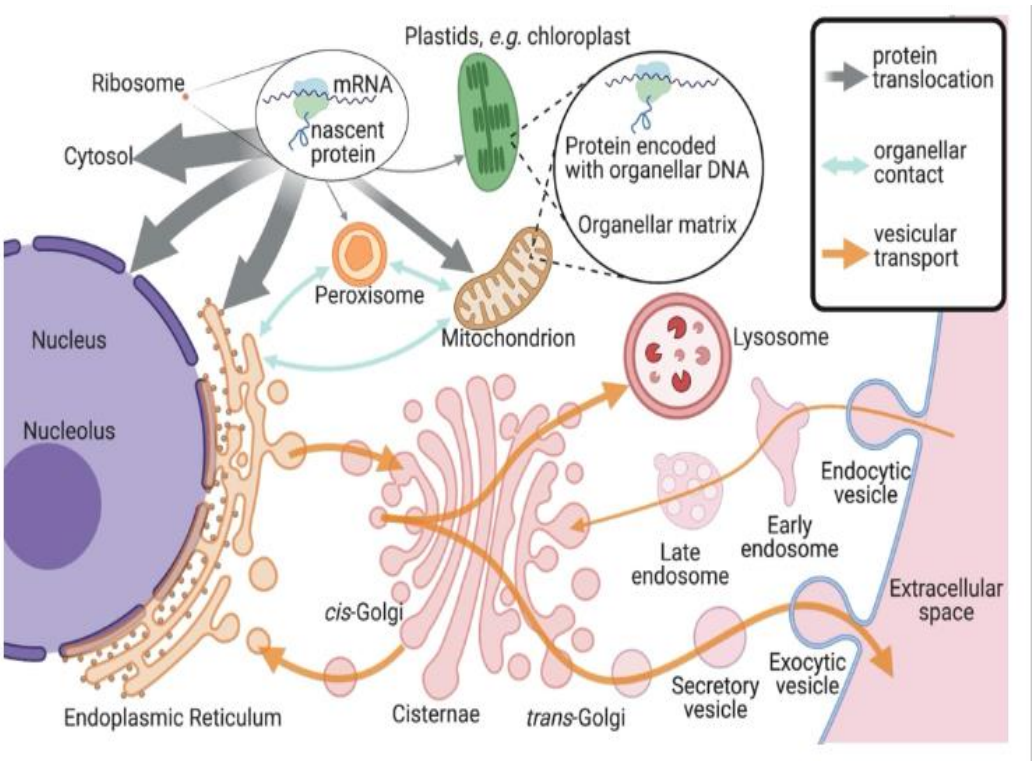

Figure 48: Pathways of proteins synthesis and sorting of plasma membrane.

Exocytosis is a general term used to denote vesicle fusion at the plasma membrane, and it is the final step in the secretory pathway that typically begins in the endoplasmic reticulum (ER), passes through the Golgi apparatus, and ends at the outside of the cell (Figure 49).

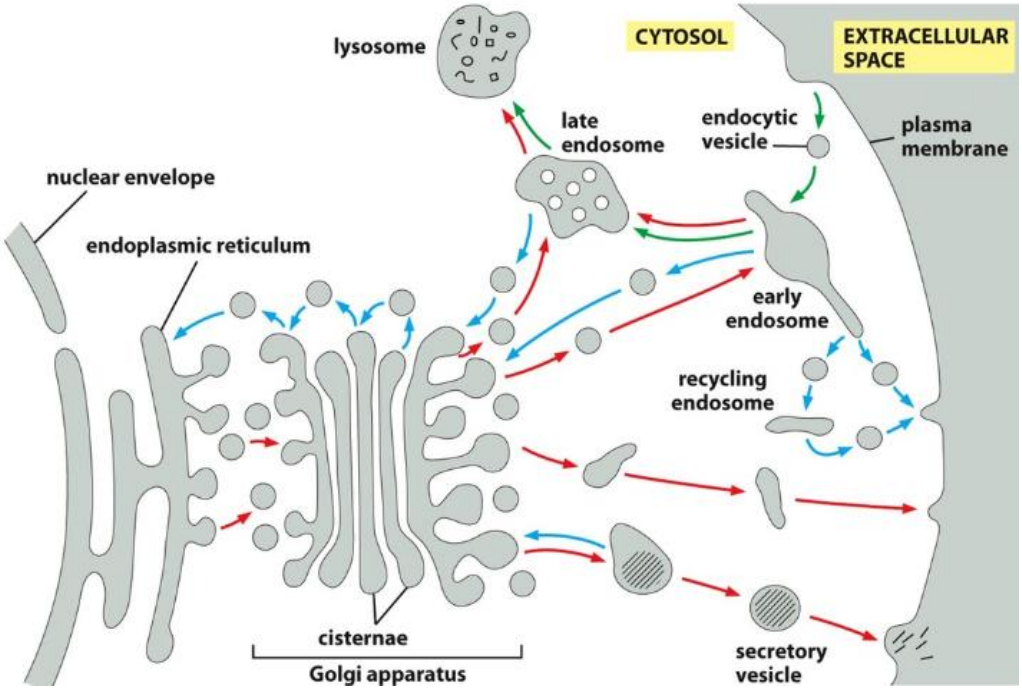

Figure 49: The secretory and endocytic pathways.

The organelles of the secretory pathway are involved in the sorting of proteins to a variety of intracellular membrane compartments and the cell surface (Figure 50). For example, proteins that are transported within the secretory pathway are either secreted from the cell, bound to the plasma membrane, sorted to lysosomes, or are retained as "residents" in any of the organelles. Within the endoplasmic reticulum (ER), newly synthesised proteins are scrutinised to ensure they are correctly folded, undergo initial glycosylation before being packaged into transport intermediates or vesicles, and then moved forward to the entry site of the Golgi apparatus. Secretory proteins are then transported through the Golgi cisternae to the *trans*-Golgi network (TGN), or Golgi exit site. At the TGN proteins are sorted according to their final destinations. The TGN is also the site where the biosynthetic and endocytic pathways converge. Molecules are internalised from the cell surface in endocytic vesicles and transported to the early endosome where extensive sorting takes place. For example, endocytosed proteins can

then be recycled to the plasma membrane (such as recycling receptors), or transported to the TGN or to the lysosome via the late endosomes for degradation. Thus, the TGN and the early endosome represent the two major sorting stations of the cell.

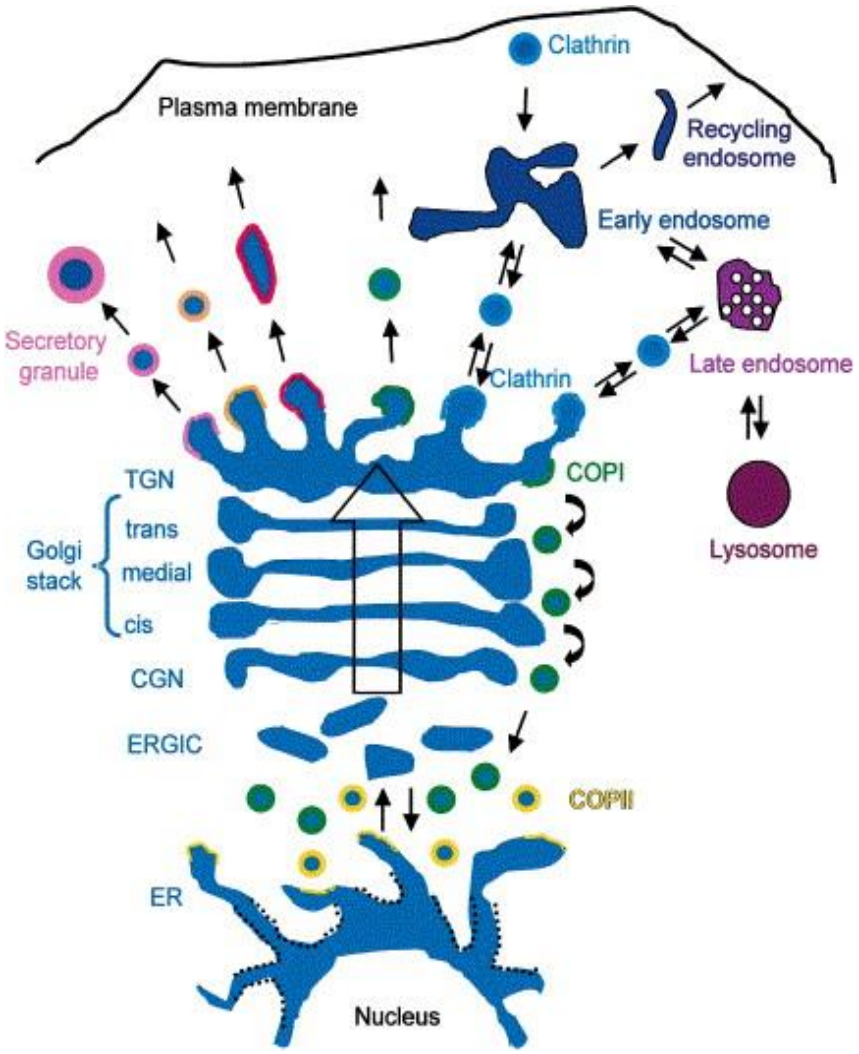

Figure 50: The secretory pathways. *Newly synthesised membrane and soluble proteins that reside in the secretory and endocytic pathways, as well as proteins that are destined for secretion, are translocated into the endoplasmic reticulum (ER), then packaged into COPII coated vesicles which fuse to become the ER-Golgi intermediate compartment (ERGIC). Numerous ERGICs merge to form the cis-Golgi network (CGN). COPI coated vesicles recycle ER proteins to and from the ERGIC and Golgi stack. Anterograde cargo moves through the Golgi stack and is sorted according to destination at the trans-Golgi network (TGN). Different types of coated vesicles and tubulovesicular carriers transport cargo to various destinations. Proteins are endocytosed at the plasma membrane and transported to the early endosome. From the early endosome proteins can be recycled to the cell surface, transported to the lysosome via the late endosome, or to the TGN.*

Exocytosis pathways (Conventional secretory pathways) are divided into constitutive secretion and regulated exocytosis or degranulation.

Conventional secretory pathway starts with cotranslational translocation of proteins containing signal peptide into the lumen of ER. Within the ER, these proteins undergo glycosylation and folding by an array of ER-resident enzymes and chaperones. Correct folding within the ER is essential, as the fate of a protein depends mostly on this step. Properly folded and glycosylated proteins are sorted into vesicular carriers and transported to the Golgi apparatus, whereas failure to achieve proper folding results in protein degradation by ER-associated degradation system. Accumulation of unfolded proteins in the ER can initiate the unfolded protein response (UPR), a compensatory mechanism to reduce protein synthesis and increase the synthesis of chaperones involved in protein folding. Post-translational processing and glycosylation continue in the Golgi until proteins achieve their physiological confirmation. From trans-Golgi network (TGN), the last site in the Golgi complex, secretory proteins can use two pathways.

The first pathway is constitutive secretion in which proteins are carried continuously by tubulovesicular carriers from the TGN to plasma membrane or endosomes (recycling endosomes then participate in constitutive secretion). This pathway is important for release of cytokines, and immune cells such as macrophages substantially up-regulate this pathway upon activation.

The second pathway is observed mainly in specialized cells, such as granulocytes, mast cells and cytotoxic T-cells, in which proteins or specialized cargos from TGN are sorted and stored in granules for secretion. Degranulation, a process of regulated exocytosis, occurs when cells are activated and cargo stored in the secretory granules can reach the plasma membrane via three routes: direct fusion of the granules with plasma membrane, transport in small vesicle to the membrane (piecemeal degranulation) or fusion of the granules with each other on the cell surface ((Figure 51).

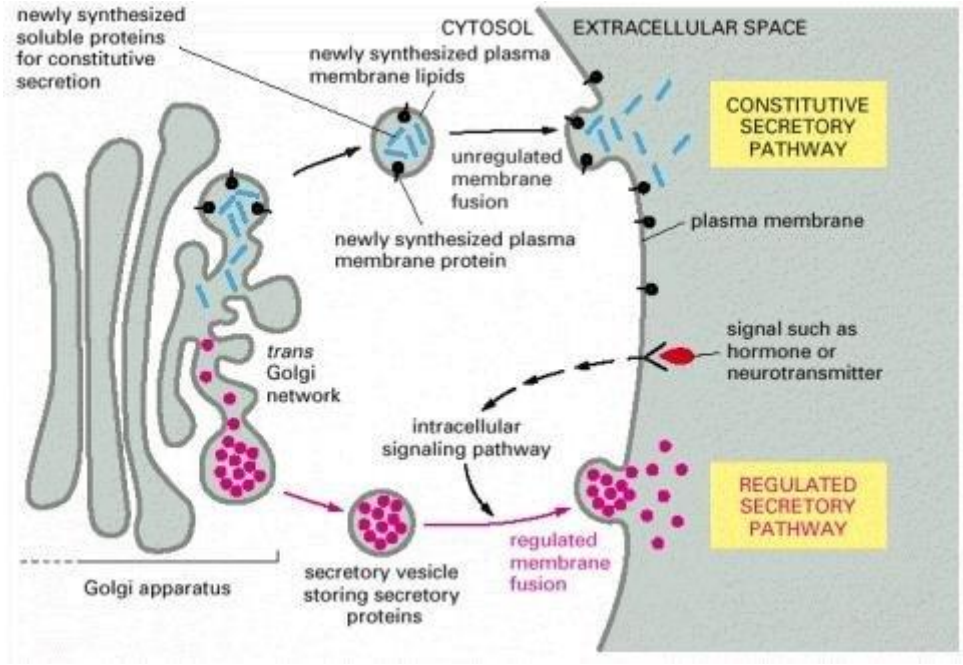

Figure 51: Constitutive and Regulated secretory pathways.

5.2.2. Endocytosis

The reverse process of exocytosis is endocytosis. During endocytosis, a region of the membrane invaginates inward (sinks) to form a pocket around the extracellular fluid, which encloses a selection of molecules or whole particles. The pocket becomes deeper and deeper until the membrane of which it is composed ruptures and becomes a closed vesicle, which is now inside the cytoplasm and which contains extracellular constituents.

All eukaryotic cells, except red blood cells, capture extracellular molecules by encasing them in vacuoles or vesicles. Cells use endocytosis to feed themselves, defend themselves and maintain their homeostasis. The liver (endocytosis of HDL in particular) and the brain (synaptic transmission) are the two organs practicing endocytosis the most. There are three modalities of endocytosis where the transported elements are of different nature and size: pinocytosis, receptor-mediated endocytosis and phagocytosis (Figure 52).

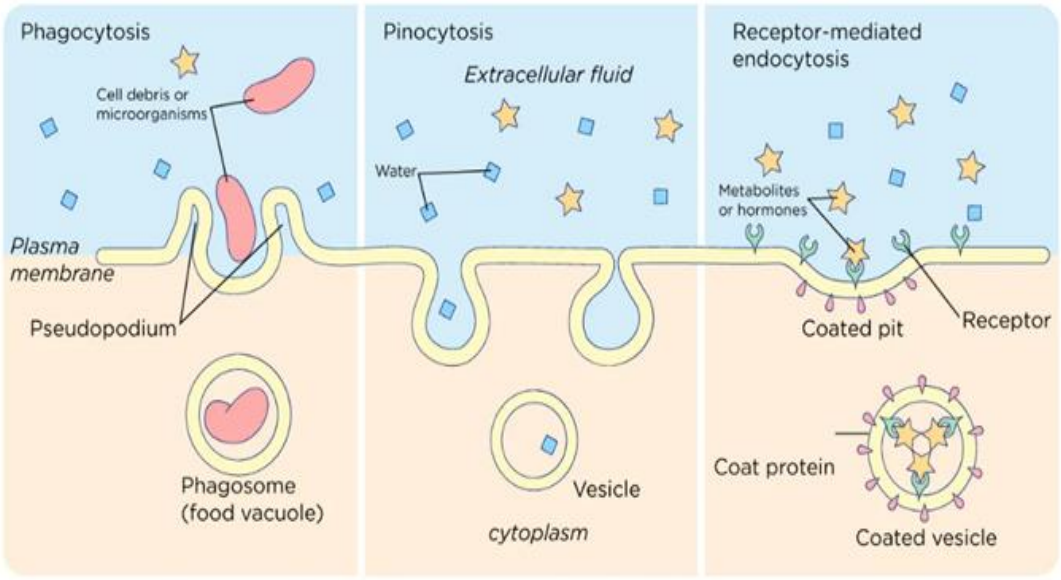

Figure 52: Three major types of endocytosis.

Pinocytosis is a non-specific form of endocytosis. During pinocytosis the cell absorbs extracellular fluid. The bud formed by the vesicle contains any solute dissolved in the fluid, however the cell does not absorb particular particles. Receptor-mediated endocytosis is a specific and highly selective process. Thanks to the presence of specific receptors in the membrane the cell can envelop and import only one type of molecule. However, phagocytosis is a large-scale endocytosis.

5.2.2.1. Pinocytosis

Pinocytosis refers to two processes, one that functions in all cells and generates small vesicles <200 nm (micropinocytosis), and one that functions under more restrictive conditions to generate large vesicles>1μm in diameter (macropinocytosis).

Pinocytosis is a form of endocytosis involving fluids containing small solutes. In humans this process occurs in cells lining the small intestine and is used primarily for absorption of fat droplets.

In endocytosis the cell plasma membrane extends and folds around desired extracellular material, forming a pouch that pinches off creating an internalized vesicle (Figure 53). The invaginated pinocytosis vesicles are much smaller than those generated by phagocytosis. The vesicles eventually fuse with the lysosome whereupon the vesicle contents are digested.

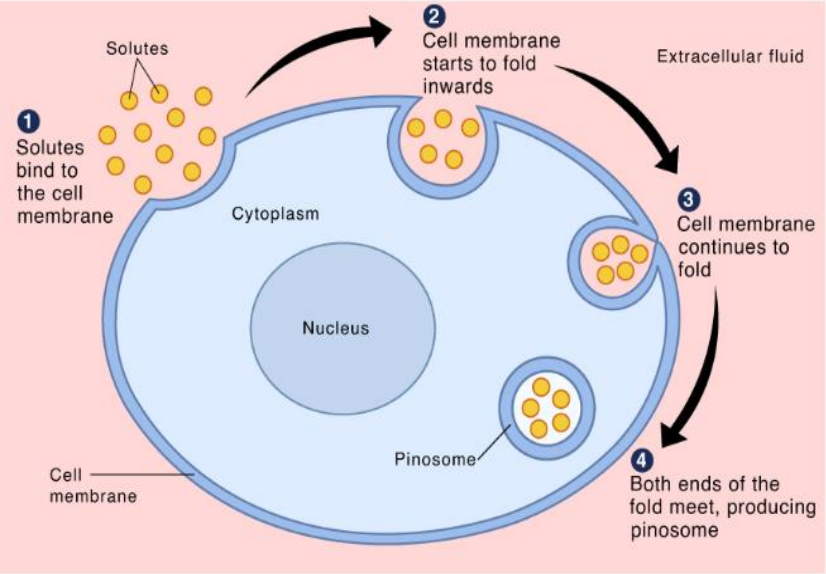

Figure 53: Different stages of pinocytosis.

Macropinocytosis occurs in many cell types, including small intestine microvilli where this process is utilized to absorb nutrients. Further, egg cells also use pinocytosis to obtain nutrients before fertilization. The macropinocytosis process is associated with various cellular processes, such as cell growth, cell proliferation, cell death, endocytosis, exocytosis, phagocytosis, chemotaxis, glycolysis, macrophage activation, and proteolysis. Furthermore, macropinocytosis is a coordinated signaling mechanism.

5.2.2.2. Phagocytosis

Phagocytosis is a complex cellular process of multicellular organisms to ingest or engulf solid large particles. In unicellular organisms, it is mostly considered as a nutritional support process for survival, and in multicellular organisms it is a vital process involved in regulation of homeostasis pertinent to cellular defense employed by immune cells.

Phagocytosis is the mechanism of internalization used by specialized cells such as macrophages, dendritic cells and polymorphonuclear neutrophils to internalize large particulate material (more than 0.5 µM), including microorganisms, apoptotic cells, or foreign substances. The internalization process begins with the stimulation of phagocytic receptors and leads to a major reorganization of the plasma membrane to extend pseudopods that will ultimately form a closed organelle, the phagosome. Upon fusion with lysosomes, the phagosome matures into a phagolysosome. The

phagolysosome is an acidic and hydrolytic formulation, leading to lysis of entities into small antigenic moieties that are transported to the cell membrane and displayed as antigens for immune cells recognition (Figure 54).

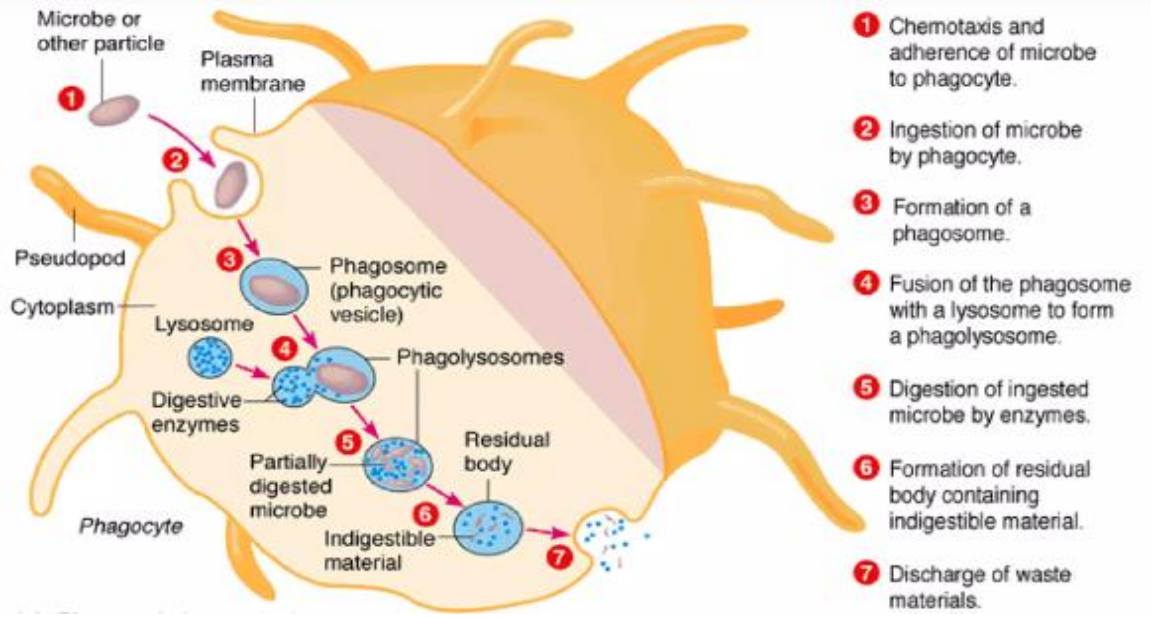

Figure 54: Mechanism of phagocytosis.

5.2.2.3. Receptor-Mediated Endocytosis

Unlike pinocytosis, which nonspecifically takes up surrounding fluid, receptor-mediated endocytosis is a highly selective process that allows cells to import, from the surrounding medium, specific large molecules that the cell needs. it is a highly regulated cellular process for internalization of various macromolecules including viral protein, toxins, metabolites, protein growth factors (EGF, PDGF), and polypeptide hormones, such as insulin and luteinizing hormone.

The macromolecules to be internalized first bind to specific cell surface receptors. These receptors are concentrated in specialized regions of the plasma membrane, called clathrin-coated pits. These pits bud from the membrane to form small clathrin-coated vesicles containing the receptors and their bound macromolecules (ligands). The clathrin-coated vesicles then fuse with early endosomes, in which their contents are sorted for transport to lysosomes or recycling to the plasma membrane.

Mechanistically, receptor-mediated endocytosis further categorized as clathrin-mediated endocytosis (CME), clathrin-independent endocytosis (CIE) (caveolin-mediated endocytosis (CavME)).

- **Clathrin-dependent endocytosis**

  Clathrin-mediated endocytosis (CME) is the major endocytic pathway for the internalization of numerous cargos. Clathrin-mediated endocytosis is a term derived from the clathrin protein, which is a key component of the endocytic process. However, in the formation of clathrin-coated endocytic vesicles, more than fifty other cytosolic proteins are involved.

  The main proteins involved in these steps are the AP2 adaptor, clathrin triskeles, the mechanoenzyme dynamin and many so-called accessory proteins with various functions, such as helping in the selective sorting of receptors or the deformation of the plasma membrane.

  Clathrin-dependent endocytosis begins with the recruitment and assembly of protein complexes called AP2 adaptors on the plasma membrane. This activity depends on GTPases. The recruited AP2 complexes group together and trigger the assembly of clathrin.

  Clathrin molecules self-assemble to form the coated pits on the cytosolic face of the membranes. The clathrin molecules are bound to the membrane via the adaptors.

  Also, the role of these adaptors is to capture transmembrane receptors in the coated pits by recognizing specific signal peptides located in the cytosolic part of the receptor.

  The receptors involved in endocytosis all have at least one transmembrane segment and an internalization motif located on their intracytoplasmic domain.These receptors concentrate in the coated wells before or only after having fixed their specific ligand.

  This process thus makes it possible to capture and concentrate in a small volume macromolecules present in very low concentrations in the lumen of the organelles or the extracellular medium.

  This endocytosis takes place in the following steps (Figure55);

a. Binding of the ligand to its receptor: Molecules present in the extracellular medium (peptide hormone, LDL, etc.) bind to the surface of the membrane at the level of specific receptors.
b. Lateral movement of the ligand-receptor complexes and grouping of these complexes: The occupied receptors then migrate in the plane of the membrane to regions covered on the cytosolic face by clathrin and adaptation proteins (AP2 adaptins). The cytosolic domain of the receptors interacts with these adaptins.
c. Formation of a clathrin-coated pit: The membrane undergoes depression and forms coated vesicles. The coating is made up of clathrin and adaptin molecules. These molecules form a hexagonal and pentagonal mesh network under the membrane. The formation of the clathrin envelope causes the invagination of the membrane which pinches to form a clathrin-coated vesicle.
d. Detachment of the vesicles: The detachment of the vesicle from the plasma membrane requires the intervention of a protein with GTPasic activity (monomeric G), dynamin. The latter forms a helical ring around the neck of the well. This ring will allow the constriction of the neck of the well and the release of the vesicle into the cytoplasm.
e. Disassembly of the clathrin coat of the newly formed vesicle: During their cytosolic transport, the clathrin-coated vesicles lose their clathrin coat under the action of the ATPase Hsc70, a chaperone protein, recruited by the auxilin protein. The components of the clathrin coat machinery are again available for a new cycle. The newly formed smooth vesicle fuses with the endosome. The endosomal environment is acidified due to the active transport of H+ ions by the ATPase H+, the acidification causes the dissociation of the ligand from its receptor.
f. Receptor recycling: The receptors are recycled to the plasma membrane from the endosomes (they can ensure several cycles of endocytosis).

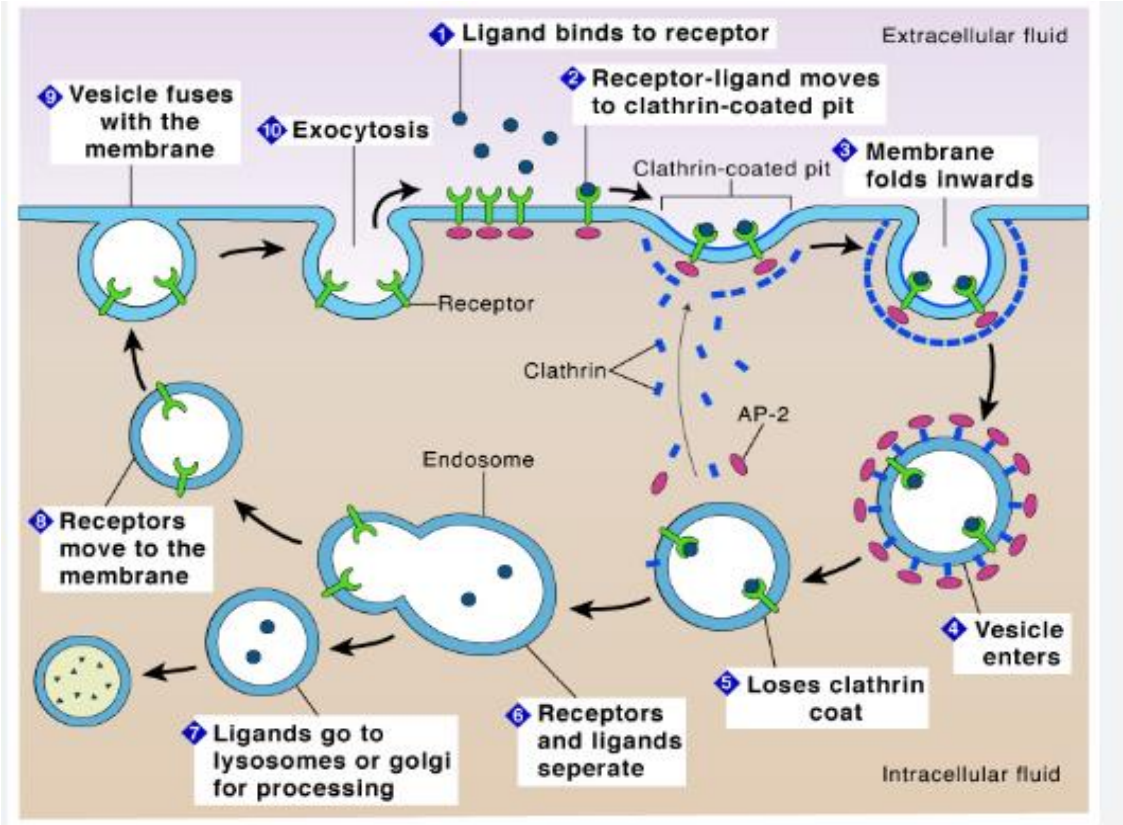

Figure 55 : Stages of clathrin-mediated endocytosis.

- **Caveolae-Dependent Endocytosis**

  Caveolae dependent endocytosis is involved in multiple biological processes, including mediating virus entry into host cells, internalizing glycophosphatidylinositol-anchored proteins and regulating certain signaling cascades.

  Caveola are cholesterol and sphingolipid-rich plasma membrane invaginations of a diameter of 60–80 nm, of which caveolin-1 is the main protein component required for caveola biogenesis.

  Caveolae form in the Golgi complex where Cav-1 oligomerizes and binds cholesterol and fatty acids, which stabilize caveolae formation. Cav-1 moves more freely in the plasma membrane, and caveolin flattens due to the decreased cholesterol levels.

  The main structural proteins of caveolae are members of the caveolin gene family, caveolin-1, caveolin-2 and caveolin-3. Morphologically defined caveolae can be formed by the expression of caveolin-1, which is a small

integral membrane protein, whose 34 hydrophobic amino acids inserted into the inner leaflet of the membrane bilayer in a special hairpin-like form and never reaches the outside of the cell. The rest of the protein is cytosolic; the N-terminal region has a special amino acid sequence functioning as scaffolding domain and has been suggested to be important for binding caveolin to cholesterol and sphingolipid-rich membrane domains. This domain is also implicated in binding to signalling molecules. The C terminus aligns along the inner leaflet of the bilayer by multiple palmitoylations.

Caveolae and caveolin-containing membrane domains on the plasma membrane have various curvatures and shapes (Ω, elongated flask, curved membrane invaginations with wide opening and narrow neck). This morphology strongly suggests that caveolae could be involved in endocytosis operating parallelly to clathrin-mediated endocytosis. In spite of the morphological evidence, it has been debated for a long time that caveolae can really pinch off from the plasma membrane. Based on morphological and biochemical data recently, it is generally accepted that caveola-mediated endocytosis functions as a true uptake mechanism parallel to the clathrin-mediated pathway. Being ligand-triggered, caveolar endocytosis provides a highly regulated way for uptake of specified substances (Figure 56).

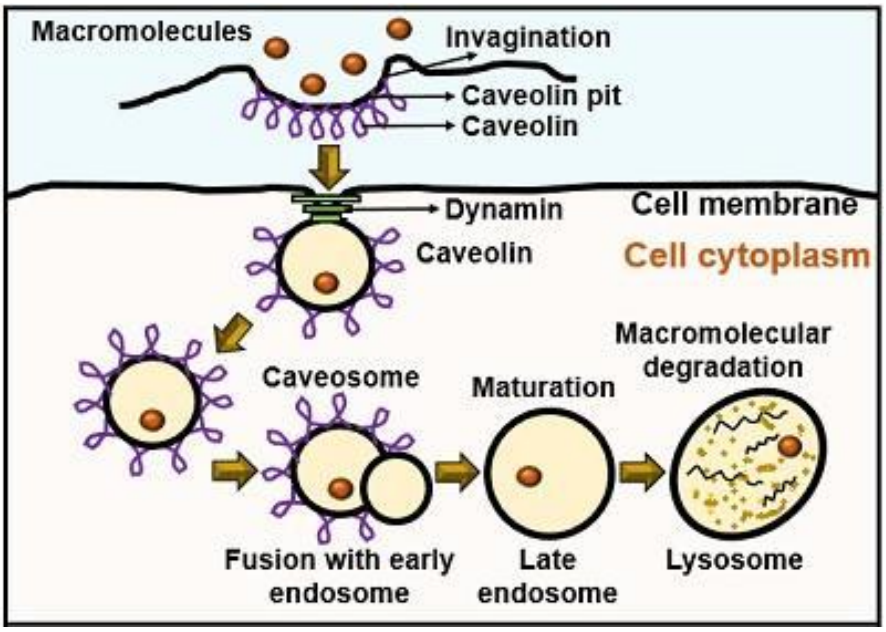

Figure 56: Stages of Caveolae-Dependent Endocytosis.

5.2.3. Endosomes and endocytic pathway

Endocytic vesicles, from clathrin-dependent or -independent pathways, fuse with elements of the endosomal compartment.

a. **Different classes of endosomes**

Endosomes are the main sorting compartments of the endocytic pathway. They are characterized by a pleiomorphic structure composed of a set of vesicles, vacuoles, tubules and multivesicular bodies. Indeed, endosomes are classified into four classes:

- Early endosomes: Newly internalized proteins are transported to early or sorting endosomes, close to the plasma membrane and which appear as a network of tubules and vacuoles.

- Recycling endosomes: Receptors that must return to the surface of the cytoplasmic membrane are accumulated in recycling endosomes which are tubular structures located in the perinuclear Golgi.

- Multivesicular bodies: Endosomal transport vesicles (vacuoles) detach from the early endosome to gradually acquire internal membrane vesicles. These elements are called multivesicular bodies.

- Late endosomes: These multivesicular bodies evolve to give late endosomes.

b. Acidification of endosomes and sorting of proteins

A proton pump, the vacuolar ATPase located in the endosome membrane, acidifies the endosome lumen. The pH of endosomes gradually changes from 6.5 in early endosomes to 5.0 in late endosomes.

The interactions of many ligands with their receptor are sensitive to pH. When the receptor-ligand complexes reach the pH threshold for their dissociation, the ligand is released into the endosome lumen while the receptor remains bound to the membrane. Soluble ligands accumulate in the endosomal vacuole while receptors go to the tubular segments. Depending on the timing of receptor dissociation from their ligand, they can be transported to the plasma membrane or to the transGolgi. Mature vacuolar segments, on the other hand, fuse directly with lysosomes where their contents are degraded.

c. **Receptor recycling and endosomes**

More than 90% of surface proteins or lipids are rapidly recycled to early endosomes via tubules or indirectly by recycling endosomes. Receptors directed to lysosomes remain in the vacuolar structures.

The sorting of receptors to be recycled occurs progressively during the maturation of the endosomes and by the cytoplasmic machinery that recovers the receptors to be recycled.

d. Late endosomes and multivesicular bodies

After the dissociation of the recycling tubules, the vacuolar part of the endosomes will migrate along the microtubules towards the perinuclear region.

The vacuoles accumulate small vesicles and tubules in their lumen by invagination of their membrane. It is the multivesicular bodies that gradually lose the remaining markers of the plasma membrane and the receptors incorporated by mistake. They gradually acquire lysosomal hydrolases by incorporating the vesicles that come from the transGolgi and become late endosomes. Late endosomes have on their internal membrane numerous mannose-6-phosphate receptors that transport lysosomal enzymes. Then the late endosomes will fuse with the lysosomes.

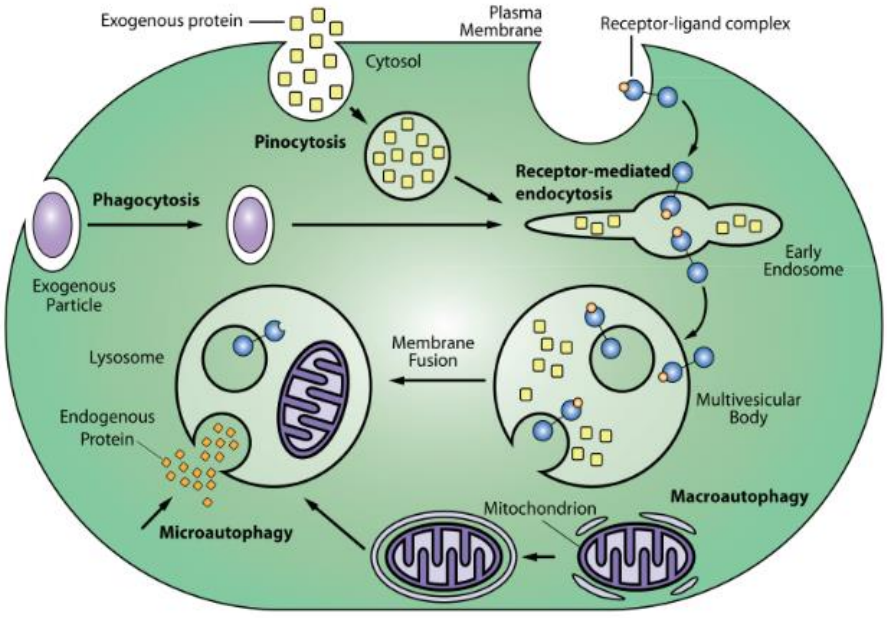

Figure 57: The endosomal pathway.

References

References

- Alhmoud, J. F., Woolley, J. F., Al Moustafa, A. E., & Mallei, M. I. (2021). DNA damage/repair management in cancers. *Advances in Medical Biochemistry, Genomics, Physiology, and Pathology*, 309-339.
- Aprelikova, O., Xiong, Y., & Liu, E. T. (1995). Both p16 and p21 families of cyclin-dependent kinase (CDK) inhibitors block the phosphorylation of cyclin-dependent kinases by the CDK-activating kinase. *Journal of Biological Chemistry*, *270*(31), 18195-18197.
- Azzwali, A. A., & Azab, A. E. (2019). Mechanisms of programmed cell death. *Journal of Applied Biotechnology and Bioengineering*, *6*(4), 156-158.
- Bai, J., Li, Y., & Zhang, G. (2017). Cell cycle regulation and anticancer drug discovery. *Cancer biology & medicine*, *14*(4), 348.
- Bhattacharya, A., Prakash, Y. S., & Eissa, N. T. (2014). Secretory function of autophagy in innate immune cells. *Cellular microbiology*, *16*(11), 1637-1645.
- Biscocho D. 2015. Phases of the Cell Cycle. *Biotechnology, Forensics, and Genetics.*
- Botchkarev, V. A., Gdula, M. R., Mardaryev, A. N., Sharov, A. A., & Fessing, M. Y. (2012). Epigenetic regulation of gene expression in keratinocytes. *Journal of investigative dermatology*, *132*(11), 2505-2521.
- Bouchier-Hayes, L., & Green, D. R. (2012). Caspase-2: the orphan caspase. *Cell Death & Differentiation*, *19*(1), 51-57.
- Braun, V., & Niedergang, F. (2006). Linking exocytosis and endocytosis during phagocytosis. *Biology of the Cell*, *98*(3), 195-201.
- Cavalcante, G. C., Schaan, A. P., Cabral, G. F., Santana-da-Silva, M. N., Pinto, P., Vidal, A. F., & Ribeiro-dos-Santos, Â. (2019). A cell's fate: an overview of the molecular biology and genetics of apoptosis. *International journal of molecular sciences*, *20*(17), 4133.
- Chao, H. X., Poovey, C. E., Privette, A. A., Grant, G. D., Chao, H. Y., Cook, J. G., & Purvis, J. E. (2017). DNA damage checkpoint dynamics drive cell cycle phase transitions. *bioRxiv*, 137307.

- Chin, M. Y., Espinosa, J. A., Pohan, G., Markossian, S., & Arkin, M. R. (2021). Reimagining dots and dashes: Visualizing structure and function of organelles for high-content imaging analysis. *Cell chemical biology*, *28*(3), 320-337.
- Chota, A., George, B. P., & Abrahamse, H. (2021). Interactions of multidomain pro-apoptotic and anti-apoptotic proteins in cancer cell death. *Oncotarget*, *12*(16), 1615.
- Chowdhury, D., & Lieberman, J. (2008). Death by a thousand cuts: granzyme pathways of programmed cell death. *Annual Review of Immunology*, *26*, 389-420.
- Civelekoglu-Scholey, G., & Cimini, D. (2014). Modelling chromosome dynamics in mitosis: a historical perspective on models of metaphase and anaphase in eukaryotic cells. *Interface Focus*, *4*(3), 20130073.
- Clarke, P. R. (1995). Cyclin-Dependent Kinases: CAK-handed kinase activation. *Current Biology*, *5*(1), 40-42.
- Connerly, P. L. (2010). How Do Proteins Move Through the Golgi.
- Cooper, G., & Adams, K. (2022). *The cell: a molecular approach*. Oxford University Press.
- De Visser, K. E., & Joyce, J. A. (2023). The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth. *Cancer cell*, *41*(3), 374-403.
- Dungrawala, H., Rose, K. L., Bhat, K. P., Mohni, K. N., Glick, G. G., Couch, F. B., & Cortez, D. (2015). The replication checkpoint prevents two types of fork collapse without regulating replisome stability. *Molecular cell*, *59*(6), 998-1010.
- Eldridge, L. (2022). Cancer cells vs. normal cells: how are they different. *Very well Health*.
- Elmore, S. (2007). Apoptosis: a review of programmed cell death. *Toxicologic pathology*, *35*(4), 495-516.
- Eşrefoğlu, M. (2019). The Golgi Apparatus: Morphology and Function with Recent Facts. *Bezmialem Science*, *7*(4), 331-8.
- Gérard, C., & Goldbeter, A. (2012). From quiescence to proliferation: Cdk oscillations drive the mammalian cell cycle. *Frontiers in physiology*, *3*, 33844.

- Gérard, C., & Goldbeter, A. (2012). From quiescence to proliferation: Cdk oscillations drive the mammalian cell cycle. *Frontiers in physiology*, 3, 33844
- Gurgis, F. M. S., Ziaziaris, W., & Munoz, L. (2014). Mitogen-activated protein kinase–activated protein kinase 2 in neuroinflammation, heat shock protein 27 phosphorylation, and cell cycle: role and targeting. *Molecular pharmacology*, 85(2), 345-356.
- Haunstetter, A., & Izumo, S. (1998). Apoptosis: basic mechanisms and implications for cardiovascular disease. *Circulation research*, 82(11), 1111-1129.
- Haunstetter, A., & Izumo, S. (1998). Apoptosis: basic mechanisms and implications for cardiovascular disease. *Circulation research*, 82(11), 1111-1129.
- He, W., Li, Z. Q., Gu, H. Y., Pan, Q. L., & Lin, F. X. (2023). Targeted Therapy of Spinal Cord Injury: Inhibition of Apoptosis Is a Promising Therapeutic Strategy. *Molecular Neurobiology*, 1-18.
- Hu, Y., Pei, R., Tao, T., & Wang, S. (2023). Progress in the study of anti-tumor drugs targeting mitochondria. *Highlights in Science, Engineering and Technology*, *74*, 1175-1181.
- Hustedt, N., Gasser, S. M., & Shimada, K. (2013). Replication checkpoint: tuning and coordination of replication forks in s phase. *Genes*, *4*(3), 388-434.
- Israels, E. D., & Israels, L. G. (2000). The cell cycle. *The oncologist*, *5*(6), 510-513.
- Iurlaro, R., & Muñoz-Pinedo, C. (2016). Cell death induced by endoplasmic reticulum stress. *The FEBS journal*, *283*(14), 2640-2652.
- Jang, T. H., & Park, H. H. (2013). PIDD mediates and stabilizes the interaction between RAIDD and caspase-2 for the PIDDosome assembly. *BMB reports*, *46*(9), 471.
- Johnson, E. S., & Kornbluth, S. (2012). Phosphatases driving mitosis: pushing the gas and lifting the brakes. *Progress in molecular biology and translational science*, *106*, 327-341.

- Jossen, R., & Bermejo, R. (2013). The DNA damage checkpoint response to replication stress: A Game of Forks. *Frontiers in genetics*, 4, 26.
- Kara, M., & Oztas, E. (2020). Endoplasmic reticulum stress-mediated cell death. *Programmed Cell Death*, 1-14
- Karçaaltincaba, M., Öztürk, M. A., & Criss, W. E. (1994). Cell cycle control Part II cyclins. *Journal of Islamic Academy of Sciences*, *7*(2), 130-136.
- Katerji, M., & Duerksen-Hughes, P. J. (2021). DNA damage in cancer development: special implications in viral oncogenesis. *American Journal of Cancer Research*, *11*(8), 3956.
- Katzman, S., Hurst-Kennedy, J., Barrera, A., Talley, J., & Higgins, R. (2020). Chapter 13: the cell cycle and its regulation in: Fundamentals of Cell Biology.
- Kiss, A. L., & Botos, E. (2009). Endocytosis via caveolae: alternative pathway with distinct cellular compartments to avoid lysosomal degradation?. *Journal of cellular and molecular medicine*, *13*(7), 1228-1237.
- Lara-Gonzalez, P., Westhorpe, F. G., & Taylor, S. S. (2012). The spindle assembly checkpoint. *Current biology*, *22*(22), R966-R980.
- Law, M. E., Corsino, P. E., Narayan, S., & Law, B. K. (2015). Cyclin-dependent kinase inhibitors as anticancer therapeutics. *Molecular pharmacology*, *88*(5), 846-852.
- Lemonnier, T., Dupré, A., & Jessus, C. (2020). The G2-to-M transition from a phosphatase perspective: a new vision of the meiotic division. *Cell Division*, 15(1), 1-17.
- Lim, Y., Dorstyn, L., & Kumar, S. (2021). The p53-caspase-2 axis in the cell cycle and DNA damage response. *Experimental & Molecular Medicine*, *53*(4), 517-527.
- Lin, Z. P., Zhu, Y. L., & Ratner, E. S. (2018). Targeting cyclin-dependent kinases for treatment of gynecologic cancers. *Frontiers in oncology*, *8*, 303.
- Lolli, G., & Johnson, L. N. (2005). CAK—cyclin-dependent activating kinase: a key kinase in cell cycle control and a target for drugs?. *Cell cycle*, *4*(4), 565-570.

- Łukasik, P., Załuski, M., & Gutowska, I. (2021). Cyclin-dependent kinases (CDK) and their role in diseases development–review. *International journal of molecular sciences*, *22*(6), 2935.
- Margalit, A., Vlcek, S., Gruenbaum, Y., & Foisner, R. (2005). Breaking and making of the nuclear envelope. *Journal of cellular biochemistry, 95*(3), 454-465.
- Meijer, L. (2003). Le cycle de division cellulaire et sa régulation. *ONCOLOGIE-PARIS-*, *5*(7/8), 311-326.
- Morgan, D. O. (2007). *The cell cycle: principles of control*. New science press.
- Obeng, E. (2020). Apoptosis (programmed cell death) and its signals-A review. *Brazilian Journal of Biology*, *81*, 1133-1143.
- Oh, N., & Park, J. H. (2014). Endocytosis and exocytosis of nanoparticles in mammalian cells. *International journal of nanomedicine*, *9*(sup1), 51-63.
- Palai, T. K., & Mishra, S. R. (2015). Caspases: an apoptosis mediator. *Journal of Advanced Veterinary and Animal Research*, *2*(1), 18-22.
- Panda, S. K., Ray, S., Nayak, S. R., Behera, S., Bhanja, S. S., & Acharya, V. (2020). A review on cell cycle checkpoints in relation to cancer. *The Journal of Medical Sciences*, *5*(4), 88-95.
- Park, H. H. (2012). Structural features of caspase-activating complexes. *International journal of molecular sciences, 13*(4), 4807-4818.
- Parrish, A. B., Freel, C. D., & Kornbluth, S. (2013). Cellular mechanisms controlling caspase activation and function. *Cold Spring Harbor perspectives in biology*, *5*(6), a008672.
- Pathak, C., Vaidya, F. U., Waghela, B. N., Jaiswara, P. K., Gupta, V. K., Kumar, A., ... & Ranjan, K. (2023). Insights of endocytosis signaling in health and disease. *International journal of molecular sciences*, *24*(3), 2971.
- Peng, A. (2013). Working hard for recovery: mitotic kinases in the DNA damage checkpoint. *Cell & bioscience, 3*, 1-8.
- Perry, J. A., & Kornbluth, S. (2007). Cdc25 and Wee1: analogous opposites?. *Cell division*, *2*(1), 1-12.

- Pluta, A. J., Studniarek, C., Murphy, S., & Norbury, C. J. (2024). Cyclin-dependent kinases: Masters of the eukaryotic universe. *Wiley Interdisciplinary Reviews: RNA*, *15*(1), e1816.
- Robert, J., & Durrieu, F. (2010). Le cycle cellulaire et sa régulation. *Cycle cellulaire et cytométrie en flux*. Editions Tec et Doc. pp :1-3.
- Ruppenthal, S. L., Noll, A., Götz, C., & Montenarh, M. (2007). Interference between p53 and cdc25C in cell cycle regulation. *International journal of oncology*, *31*(2), 345-362.
- Rush, J. S. (2015). Role of flippases in protein glycosylation in the endoplasmic reticulum. *Lipid insights*, *8*, LPI-S31784.
- Schatten. H. (2013). Mitosis. H. Schatten, in Brenner's Encyclopedia of Genetics (Second Edition), 2013. Pages 448-451
- Schwarz, D. S., & Blower, M. D. (2016). The endoplasmic reticulum: structure, function and response to cellular signaling. *Cellular and molecular life sciences*, *73*, 79-94.
- Shackelford, R. E., Kaufmann, W. K., & Paules, R. S. (1999). Cell cycle control, checkpoint mechanisms, and genotoxic stress. *Environmental health perspectives*, *107*(suppl 1), 5-24.
- Shibata, Y., Voeltz, G. K., & Rapoport, T. A. (2006). Rough sheets and smooth tubules. *Cell*, *126*(3), 435-439.
- Silva, P. M., & Bousbaa, H. (2022). BUB3, beyond the Simple Role of Partner. *Pharmaceutics*, *14*(5), 1084.
- Sinha, T. (2018). Tumors: benign and malignant. *Cancer Therapy & Oncology International Journal*, *10*(3), 52-54.
- Sladky, V. C., & Villunger, A. (2020). Uncovering the PIDDosome and caspase-2 as regulators of organogenesis and cellular differentiation. *Cell Death & Differentiation*, *27*(7), 2037-2047.
- Smith, S. M., & Smith, C. J. (2022). Capturing the mechanics of clathrin-mediated endocytosis. *Current Opinion in Structural Biology*, *75*, 102427.
- Solano-Gálvez, S. G., Abadi-Chiriti, J., Gutiérrez-Velez, L., Rodríguez-Puente, E., Konstat-Korzenny, E., Álvarez-Hernández, D. A., ... & Vázquez-López, R.

(2018). Apoptosis: activation and inhibition in health and disease. *Medical Sciences, 6*(3), 54

- Sonawane, G. B. *Chapter-1 Biology of Normal Cell and Cancer Cell* (Doctoral dissertation, Department of Pharmacy, Suresh Gyan Vihar University).
- Tripathi, Vidisha, and Kannanganattu V. Prasanth. "Cell nucleus." *eLS* (2011). eLS & 2011, John Wiley & Sons, Ltd. www.els.net.
- Ullmann G. M . (2001). Chapter 6 - Charge Transfer Properties of Photosynthetic and Respiratory Proteins. *Supramolecular Photosensitive and Electroactive Materials*. *2001,* 525-584.
- van Anken, E., & Sitia, R. (2016). The endoplasmic reticulum. *Ralph A. Bradshaw, Philip D. Stahl. Encyclopedia of Cell Biology.(Academic Press, 2016),* 156-167.
- Van Vliet, C., Thomas, E. C., Merino-Trigo, A., Teasdale, R. D., & Gleeson, P. A. (2003). Intracellular sorting and transport of proteins. *Progress in biophysics and molecular biology*, *83*(1), 1-45.
- Walczak, C. E., Cai, S., & Khodjakov, A. (2010). Mechanisms of chromosome behaviour during mitosis. *Nature reviews Molecular cell biology, 11*(2), 91-102.
- Wang, J.J., Lei, K.F., & Han, F.J.E.R.M.P.S. (2018). Tumor microenvironment: recent advances in various cancer treatments. *European Review for Medical & Pharmacological Sciences*, *22*(12).
- Wang, Y., & Seemann, J. (2011). Golgi biogenesis. *Cold Spring Harbor perspectives in biology*, *3*(10), a005330.
- Wilson, C., Venditti, R., Rega, L. R., Colanzi, A., D'Angelo, G., & De Matteis, M. A. (2011). The Golgi apparatus: an organelle with multiple complex functions. *Biochemical Journal*, *433*(1), 1-9.
- Wong, R. S. (2011). Apoptosis in cancer: from pathogenesis to treatment. *Journal of experimental & clinical cancer research, 30,* 1-14.
- Zhang Y. (2023) Exploring the endoplasmic reticulum: Structure and function. *American Journal of Translational Research, 4* (7).

- Zhang, P., Zhang, X., Liu, X., Khan, S., Zhou, D., & Zheng, G. (2020). PROTACs are effective in addressing the platelet toxicity associated with BCL-XL inhibitors. *Exploration of targeted anti-tumor therapy*, *1*(4), 259.
- https://fr.slideshare.net/slideshow/phagocytosis-238691143/238691143
- https://facts.net/science/biology/8-intriguing-facts-about-protein-trafficking-and-sorting/#google_vignette
- https://jackwestin.com/resources/mcat-content/plasma-membrane/exocytosis-and-endocytosis
- https://alg.manifoldapp.org/api/proxy/ingestion_sources/f9abe6c2-a2f0-4a2f-a89f-50b0ffc3131b
- https://basicmedicalkey.com/cell-death/#c08fig0004xa
- https://byjus.com/neet/plasma-membrane-structure/
- https://fire.biol.wwu.edu/cmoyer/zztemp_fire/biol205_S07/lec05.pdf
- https://propolis-snv.weebly.com/uploads/4/7/2/3/47235901/diapos_c.pdf
- https://sciencenotes.org/prokaryotic-vs-eukaryotic-similarities-and-differences/
- https://uomustansiriyah.edu.iq/media/lectures/6/6_2018_09_30!02_53_03_PM.pdf
- https://www.creative-diagnostics.com/extrinsic-apoptosis-pathway.htm
- https://www.easybiologyclass.com/intrinsic-pathway-of-apoptosis-apoptosis-molecular-mechanism-part-1/
- https://www.khanacademy.org/science/ap-biology/cell-communication-and-cell-cycle/regulation-of-cell-cycle/a/cell-cycle-regulators
- https://www.medparkhospital.com/en-US/disease-and-treatment/differences-between-cancer-cells-and-normal-cells.
- https://www.ncbi.nlm.nih.gov/books/NBK9958/
- https://www.ptglab.com/products/featured-products/cell-cycle-and-checkpoint-controls/
- https://www.researchgate.net/figure/Cyclin-CDK-regulation-of-the-mammalian-cell-cycle-The-cell-cycle-consists-of-a-DNA_fig1_42587723