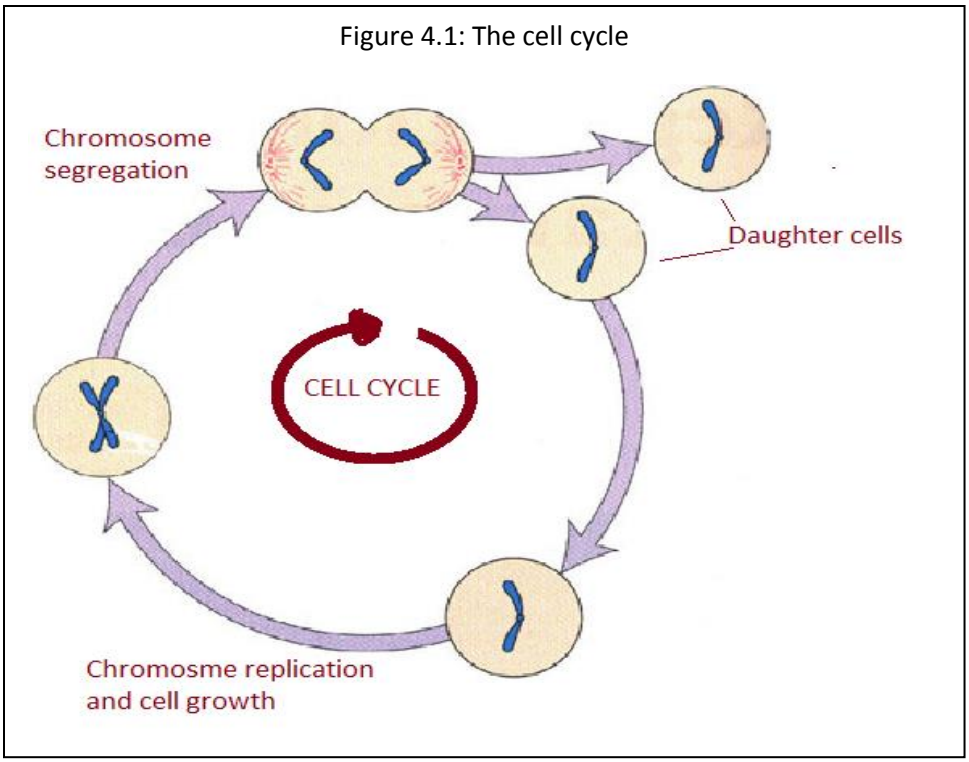
## CELL CYCLE AND ITS REGULATION

" Where a cell arises, there must be a previous cell, just as animals can only arise from animals and plants from plants." This is the cell doctrine, proposed by the German Pathologist Rudolf Virchow in 1858. This is achieved by way of cell division. All living organisms, right from bacteria to multicellular animals are products of cell growth and division whose beginning extends back to 3 billion year ago. Cells reproduce by initially duplicating their content and then dividing into two. This cycle of duplication and division is called cell cycle.in unicellular organisms, cell cycle results in the production of a complete new organism, while in multicellular organisms, it is highly complex and is essential for functioning of the organism. Millions of cells are to be synthesized in us to replace dead cells for our survival.

Certain characteristics of cell cycle are however universal, i.e., to pass the genetic information to the next generation of cells. For this the DNA in each chromosome is first replicated to produce identical copies, and then these duplicated copies of chromosomes are to be segregated equally to the daughter cells (Figure 4.1). However eukaryotes have evolved a complex system comprising of many proteins to regulate the cell cycle which is known as the "Cell-cycle control system". It comprises of biochemical switches that control main events of cell cycle from signals both from inside as well as outside the cells. It involved controlling progression inside the cells and at times delays progression into next phase until the previous events are completed. If this cell-cycle control system fails, it can result in cancer.

Figure 4.1: The cell cycle



**An overview of Cell Cycle:**

The basic function of Cell cycle is to duplicate DNA in the chromosome accurately and its segregation into two genetically identical daughter cells. Hence cell cycle has two important phases: S-phase (synthetic phase) during which DNA duplication occurs and it occupies about half of the cell cycle in a typical mammalian cell. After S-phase, chromosome segregation and cell division occurs in M-phase (M for Mitosis) which requires much less time.

M-phase involves packaging of duplicated DNA into chromosomes, their condensation, and break down of nuclear envelope and replicated copes of chromosomes appear each consisting of a pair of sister chromatids. These are found attached to mitotic spindle. As mitosis proceeds during metaphase, chromosomes get aligned at the equator of the mitotic spindle, poised for segregation. In Anaphase sudden separation of sister chromatids takes place, where they move to opposite poles. This is followed by decondensation of chromosomes and reformation of Nuclei. The cells are now pinches to undergo division of cytoplasm (cytokinesis) which completes cell division (Figure: 4.2).

Before duplication of DNA, a much longer gap exists for growth and accumulation of proteins which is called as gap phase. This includes $G_1$ phase and $G_2$phase, where in $G_1$ is between M phase and S phase and $G_2$ is between S phase and mitosis (Figure 4.3). Thus eukaryotic cell cycle comprises of four sequence of phases: $G_1$, S, $G_2$ and M phases. The $G_1$, S and $G_2$ together are called the interphase. A typical human cell cycle, if it occurs in 24 hours, interphase occupies 23 hours and mitosis occurs in 1 hour. If extracellular conditions are unfavorable, the cell cycle is delayed and cells enter into a specialized resting state known as $G_o$ phase which can be for few hours, weeks to years, until the onset of favorable conditions

Figure 4.2: Events of Eukaryotic cell division

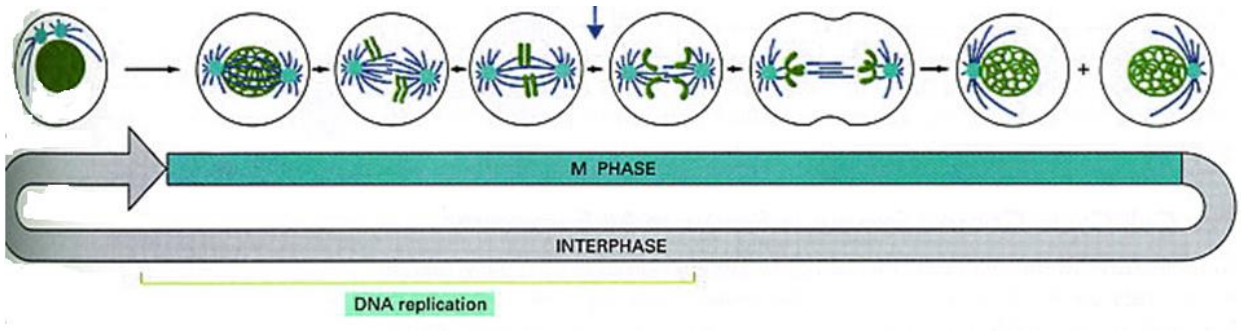
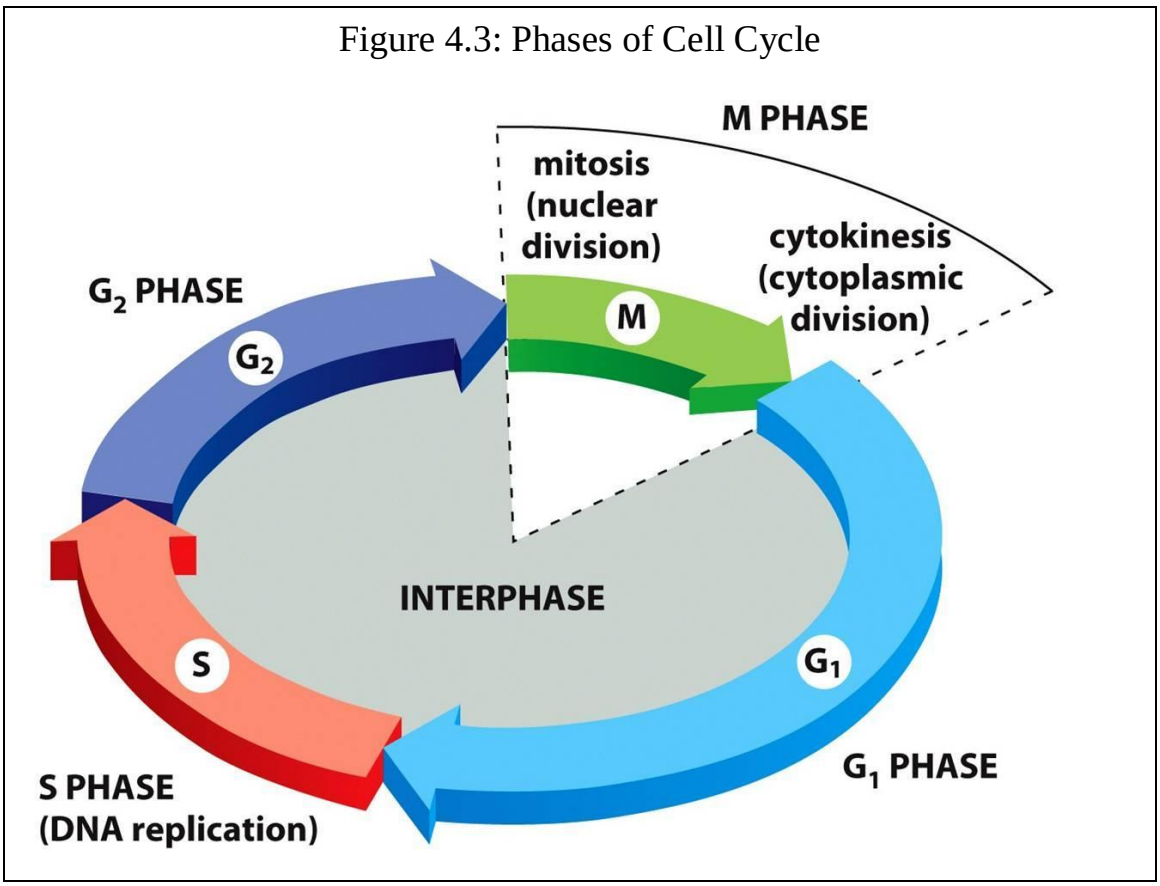


Figure 4.3: Phases of Cell Cycle



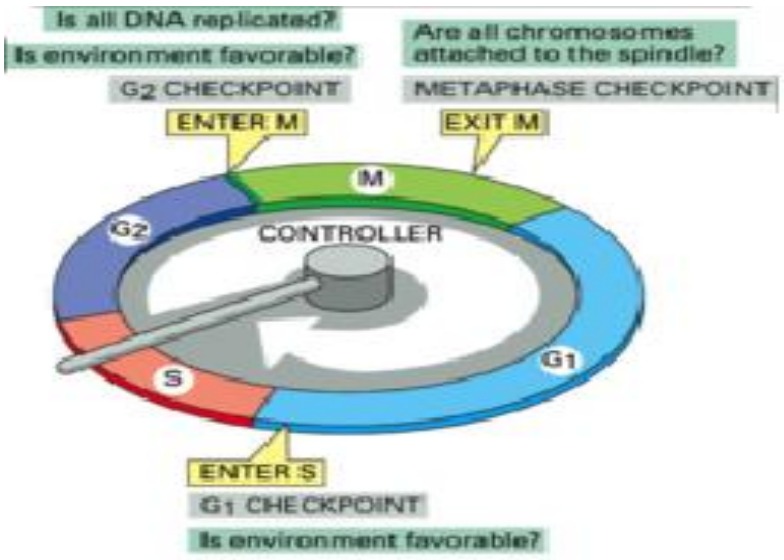

**Components of Cell Cycle control system:**

The cell cycle control system operates much like the control system of a washing machine. This control system in cells must have the following features.

- A clock that turns on each event at a specific timer this providing a fixed amount of time for the completion of each event
- A mechanization for initialing events in the correct order i.e., entry into mitotic phase after DNA replication is over.
- Mechanization to ensure that each stage is triggered only once for cycle.
- Binary (on/ off) switches thattrigger events in a complete, irreversible fashion.
- Robustness. Backup mechanism to ensure that the cell cycle works properly even when parts of the system mall function.
- Adaptability so that the system behavior can be modified to suit specific cell types or environmental conditions.
- Hence a cell cycle has several control systems to monitor its smooth preogression.

**1) Check point: T**hese are the points at which cycle can be arrested if previous events have not been completed. The two prominent check points are the $G_1$check point, the $G_2$ check point and metaphase check point (Figure 4.4).

Figure 4.4: The control of the Cell cycle




Progression into M phase is delayed at $G_1$ and $G_2$ check point if DNA is damaged by radiation or chemicals. At this stage of delay DNA is repaired and then released for next phase for progression. Metaphase check point operates through next intracellular signals. It maintains attachment mitotic spindle to chromosomes. A cell has many chromosomes, and if chromosome sends a positive signal to cell cycle control system after attachment of mitotic spindle is attached. It becomes difficult to identify the fraction of positive signal to be sent by a chromosome left out with spindle unattached. On the other hand, if unattached chromosome send a negative signal to inhibit progression of cell cycle, it will be easily detected by the cell cycle and its progression stops.

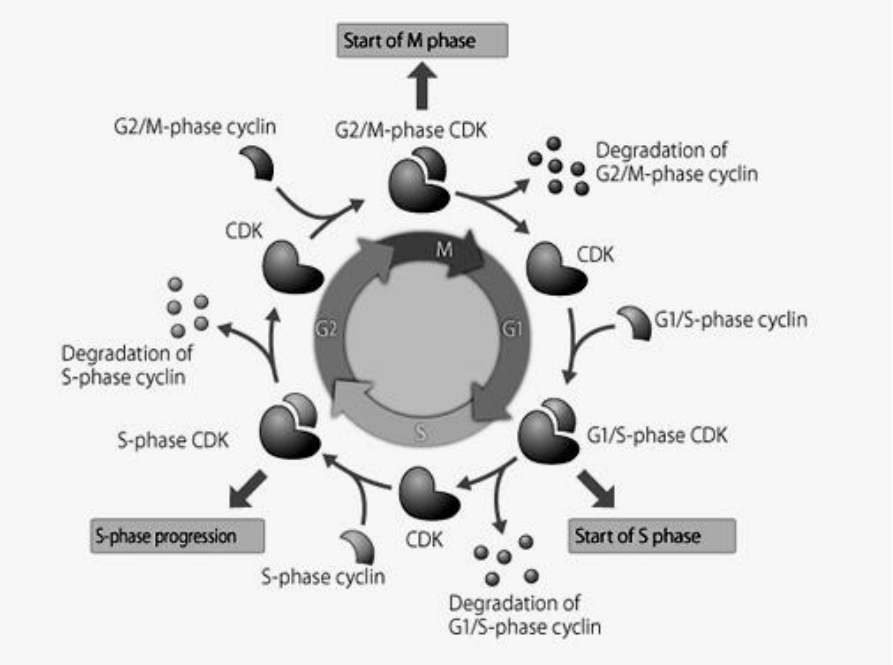
**2) Cyclically activated protein kinase:**

The heart of cell cycle is the family of protein kinases known as Cyclin-dependent kinases (Cdks). Activity of these rises and falls as the cell progresses through the cell cycle, by their phosphorylation. They mainly regulate DNA replication, mitosis and cytokinesis. Eg:

Increased phosphorylation of Cdks leads to chromosome condensation, nuclear envelope break down and spindle assembly before mitosis.

Cyclical changes in Cdk activity are controlled by a complex array of enzymes and other proteins. Most important of Cdk regulators are cyclins. These are named so because they undergo a cycle of synthesis and degradation in each cell cycle while Cdk levels remain constant. Cyclical changes in cyclin levels result in the cyclic assembly and activation of cyclin-Cdk complexes and this activation triggers Cell-cycle events (Figure: 4.5).

Figure 4.5: Simplified view of cell cycle control system by Cdks




There are four classes of cyclins each defined by the stage of the cell cycle which they bind Cdks and function. Three of these classes are required in all eukaryotic cells:

1. **$G_1$/S – cyclins:** They bind Cdks at the end of $G_1$ and commit the cell to DNA replication.
2. **S- cyclins:** they bind Cdks during S phase and are required for the initiation of DNA replication.
3. **M-cyclins:** they promote the events of mitosis.

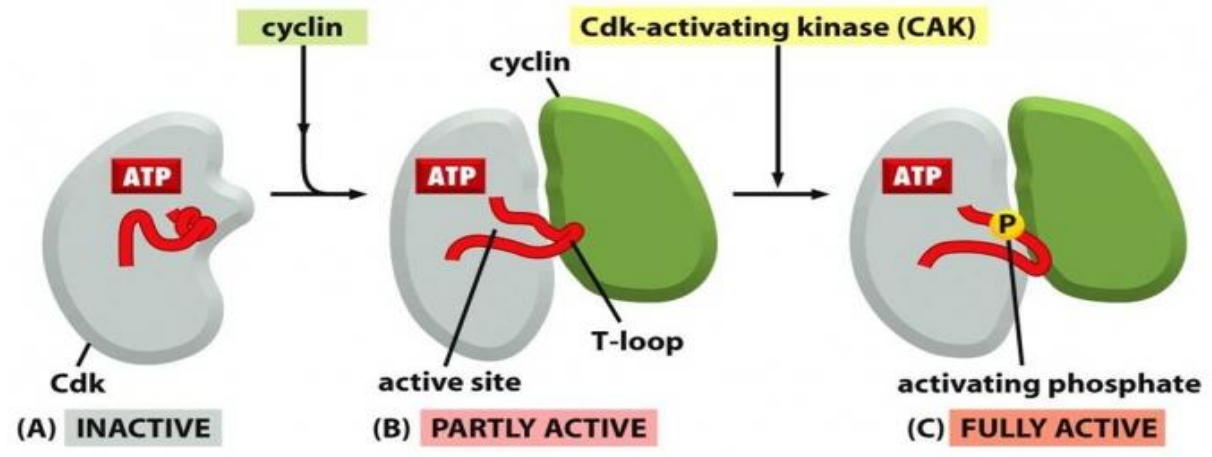
The fourth class of cyclins, the $G_1$ – cyclins, helps promote passage through the start or the restriction point in late $G_1$ phase. In yeast cells, a single Cdk protein binds all classes of

cyclins, while in vertebrates there are four Cdks. Two interact with $G_1$-cyclins, one with $G_1$/S and S-cyclins and one with M-cyclin (Table -1).

| Cyclin- dependent Kinases | Vertebrates | |
|---|---|---|
| | Cyclins | CDK partner |
| $G_1$-Cdk | Cyclin D | Cdk4, Cdk6 |
| $G_1$/S Cdk | Cyclin E | Cdk2 |
| S Cdk | CyclinA | Cdk2 |
| M Cdk | Cyclin B | Cdk 1 |
| | | |
| | | |

The activity of Cdk in association with cyclins is revealed by studying its 3d structure. In this absence of Cdk, cyclins exist in an inactive state and the active site region is blocked by a region of protein ( Eg: T-loop in Cdk2). Binding of cyclin causes T-loop to move out of the active site, resulting in its partial activation. While phosphorylation by Cdk- activating kinase (CAK) at a threonine residue near the entrance of Cdk active site improves the ability of Cdk to bind to its target site (figure 4.6).

Figure 4.6: Structural basis of Cdk activation



**Suppression of Cdk activity:**

Additional mechanism exists to regulate Cdk activity during cell cycle. The activity of cyclin-Cdk complex can be inhibited by phosphorylation at a pair of amino acids in the roof of its active sire by a protein kinase known as Wee 1. While dephosphorylation of the same by a Phosphatase known as Cdc25 increases Cdk activity (Figure 4.7). the cyclin-Cdk complex activity can also be regulated by the binding of Cdk inhibitor proteins (CKIs). These are primarily employed in control of $G_1$ and S phase and they dramatically rearrange the structure of Cdk active site (figure 4.8).

Figure 4.7: Regulation of Cdk activity of cell activity by inhibitory phosphorylation

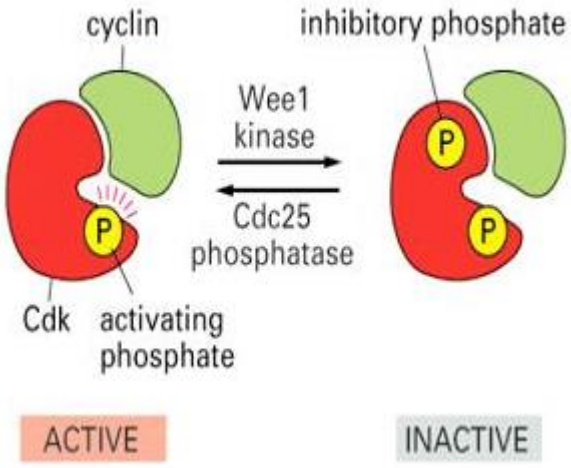
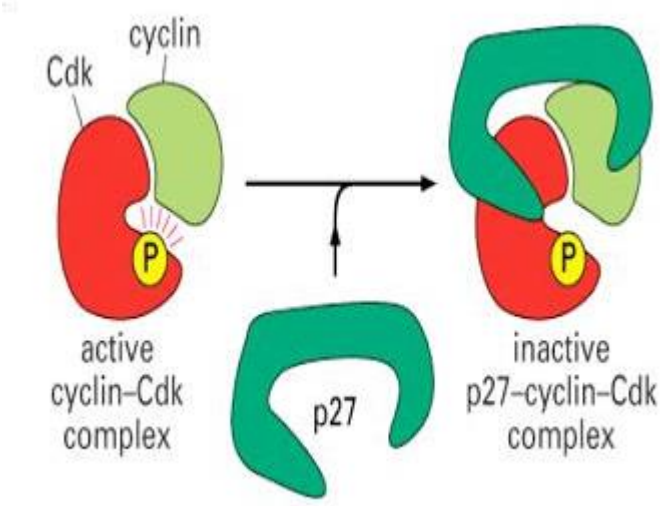
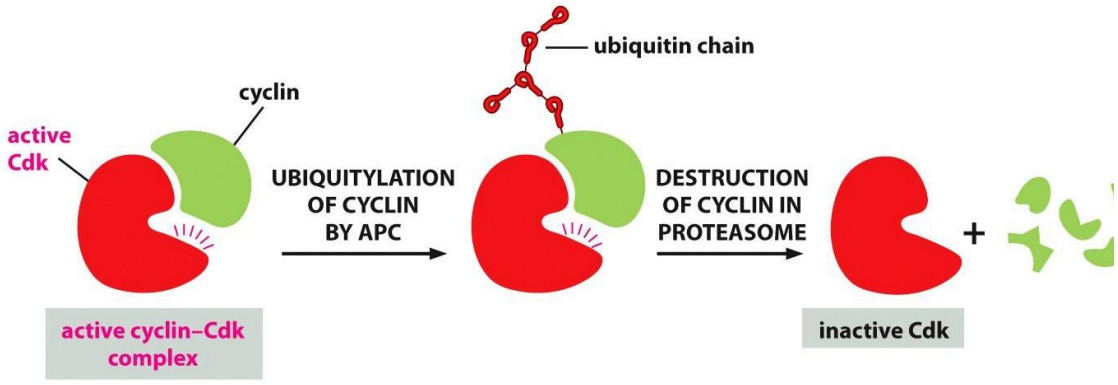



Figure 4.8: Inhibition of cyclin-Cdk complex by CKI



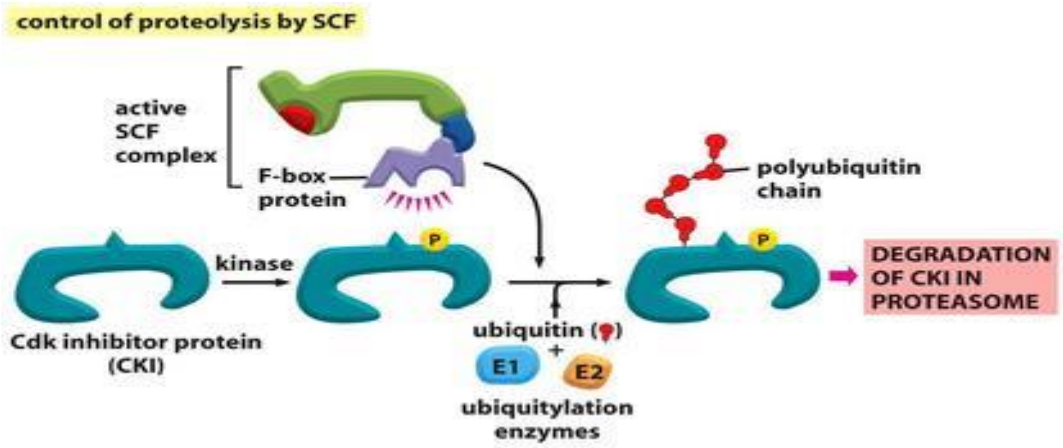
**3) Cyclical Proteolysis:** Cell cycle control depends on atleast two distinct enzyme complexes that act at different times in the cell cycle to cause proteolysis of key proteins and there by inactivating them. The cyclins are proteolyzed by a Ubiquitin-dependent mechanism, like that involved in the proteolysis of many other intracellular proteins. An activated enzyme complex recognizes specific Amino acid sequences on the cyclin and attaches multiple copies of Ubiquitin to it, thereby marking the protein for complete destruction in Proteosomes. The rate-limiting step in cyclin destruction is the final Ubiquitin- transfer reaction catalyzed by enzymes known as Ubiquitin ligases (figure 4.9).

Figure 4.9: Ubiquitin based proteolysis of cyclins

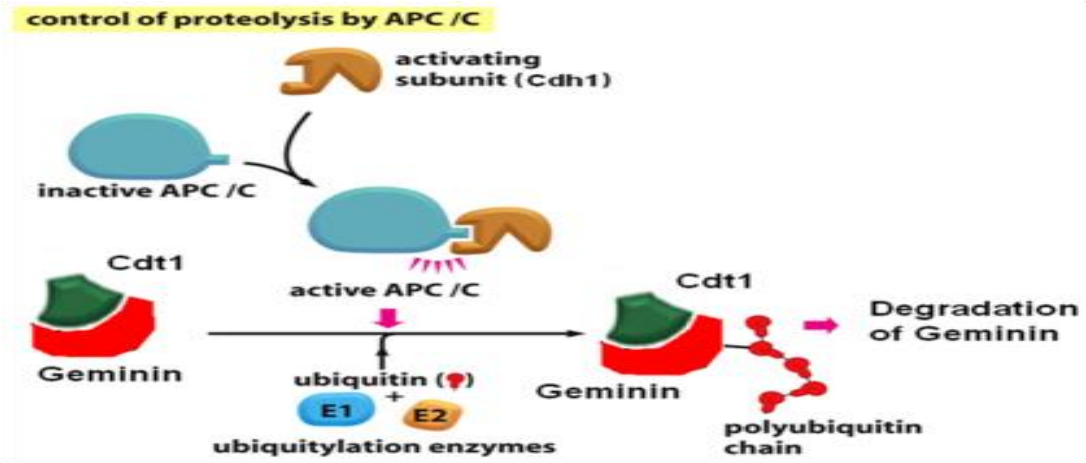



Two Ubiquitin ligases are important in the destruction of cyclins and other cell regulations. In $G_1$and S phase, an enzyme complex called SCF is responsible for ubiquitylation and destruction if $G_1$/S-cyclins and certain CKI proteins that control S – phase initiation. In M-phase, the anaphase-promoting complex (APC) is responsible for Ubiquitylation and proteolysis of M-cyclins and other regulators of histones (Figure 4.10). APC has two large multi subunit complexes, containing same related components as SFC, but they are regulated in different ways. SCF activity is constant during the cell cycle. Ubiquitylation by SCF is controlled by changes in the phosphorylation state of its target proteins. Only specific phosphorylated proteins are recognized, Ubiquitylated and destroyed by APC activity, by contrast changes at different stages in the cell cycle. APC is turned on mainly by the addition of activating subunits to the complex.

Figure 4.10: Control of Proteolysis by SCF and APC during cell cycle




SCF along with E1 and E2 serves as Ubiquitin ligase




APC activated by Cdc20 related proteins along with E1 and E2 acts as ubiquitin ligase

## INTRACELLULAR CONTROL OF CELL – CYCLE EVENTS

Each of the different Cyclin- Cdk complexes serves as a molecular switch that triggers specific cell cycle event.

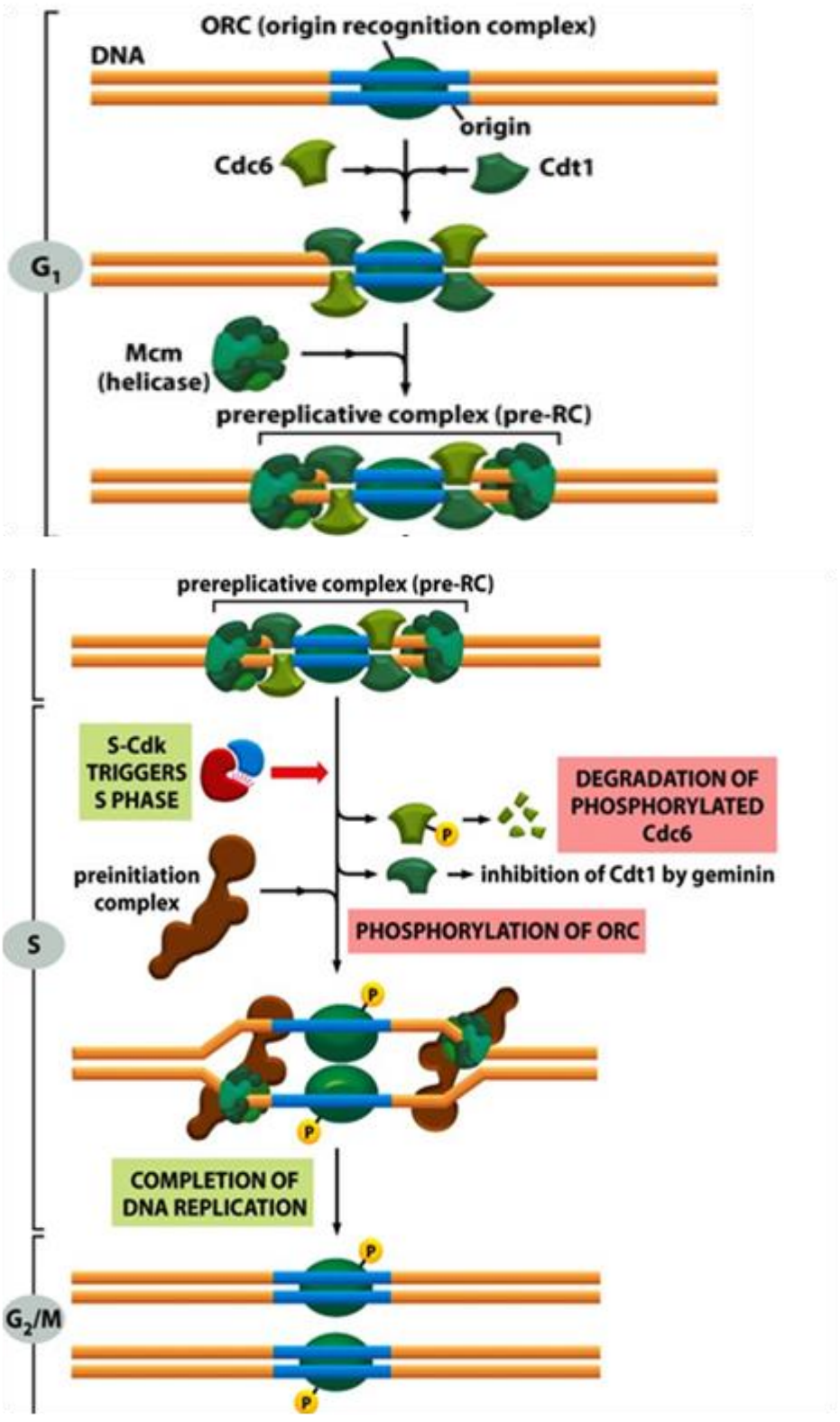
1) **S-phase cyclin-Cdk complexes (S-Cdks) initiate DNA replication once per cycle:**

Studies in yeast have revealed that DNA replication begins at Origins of replication. A large, multiprotein complex known as The Origin Recognition Complex (ORC), which binds to Origin of replication and served as landing pad for several other regulatory proteins was identified.

ORC remains associated with the replication origin throughout the cell cycle. In early G phase, Cdc associates with ORC. Aided by Cdc6, Mcm ring complexes then assemble on the adjacent DNA resulting in the formation of the pre-replicative complex. The S-Cdk triggers origin firing, assembling DNA polymerase and other replications proteins and activating the MCM protein rings to migrate along DNA strands as DNA helicase. The S-Cdk also blocks re-replication causing the deassociation of Cdc 6 from origins, its degradation, and the export of all excess MCM out of nucleus. Cdc 6 and MCM cannot return to reset an ORC-containing origin of another round of DNA replication until M-Cdk has been inactivated towards the end of mitosis (Figure 4.11).

Thus several cyclin- Cdk complexes cooperate to restrain pre-RC assembly and prevent DNA replication.

Figure 4.11: The initiation of DNA replication once per cell cycle by Cdc6 and Mcm rings



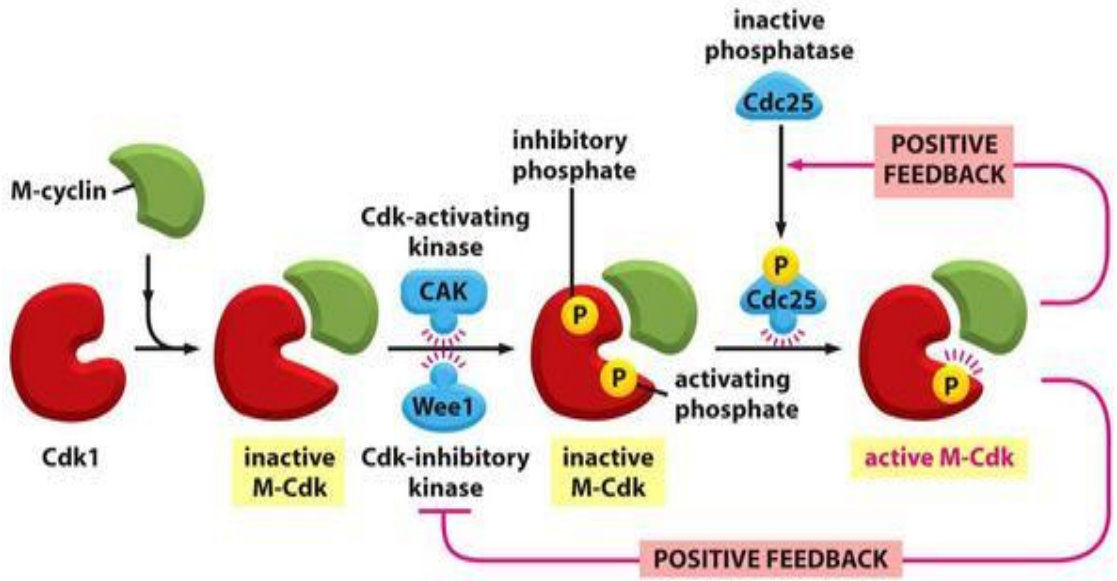
**2) The Activation of the M-phase Cyclin-Cdk complexes (M-Cdks) triggers entry into matrix:**

The events of mitosis are triggered by M-Cdks, which are activated after S-phase is completed. Activation of M-Cdk begins with the accumulation of M-Cyclin after its synthesis during $G_2$ phase. Thus accumulation leads to gradual accumulation of M-Cdk as the cell approaches mitosis. This inactive M-Cdk stock pile is activated in late $G_2$ phase by protein phosphatase Cdc25, which removes inhibitory phosphates from inactive M-Cdks. At the same time activity of inhibitory kinase Wee1 activity is suppressed ensuring M-Cdk to gain activity. Two protein kinases activate Cdc25:

1) Polokinase that phosphorylates Cdc25 at the end of the sites, and
2) Activating kinase of M-Cdk itself, which phosphorylates a different set of sites of Cdc 25. M-Cdk also phosphorylates and inhibits Wee 1 (figure 4.12).

The ability of M-Cdk to activate its own activation (Cdc25) and inhibit its own inhibitor (Wee1) indicates that M-Cdk activation in mitosis involves a positive feedback loop.

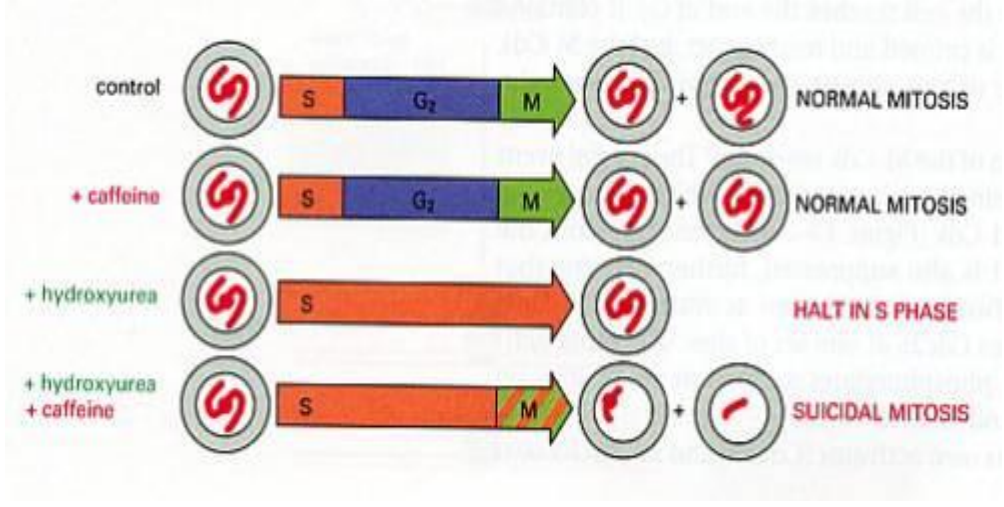
Figure 4.12: Activation of M-Cdk



**3) The DNA replication check Point:**

Entry into mitotic phase is blocked by incomplete DNA replication, else it would be a disaster leading to inheritance of incomplete sets of chromosomes by the daughter cells. Sensor mechanisms of unknown molecular nature, detect either the unreplicated DNA or the corresponding unfinished replication forks and send a negative signal to the cell cycle control system, blocking the activation of M-Cdk.

Figure 4.13: DNA replication in cells treated with caffeine and Hydroxyl urea indicating S phase check point




This figure shows studies of mammalian cells treated with caffeine and Hydroxylurea either alone or in combination. This block activities of Hydroxy urea (stops DNA synthesis) activities. Check point mechanism that arrests cells in S phase delaying mitosis. But if caffeine is added with hydroxyl urea, check point mechanism fails, cells proceed into mitosis and results in incomplete DNA replication. The final targets of the negative check point signals are the enzymes that control M-Cdk activation. The negative signal activates a protein kinase that inhibits Cdc25 protein phosphatase. As a result, M-Cdk remains phosphorylated and inactive until DNA replication is completed.

**4) M-Cdk prepares the Duplicated chromosomes for separation:**

M-Cdks bring about diverse functions and complex arrangements that occur in the early stages of mitosis. M-Cdk must induce the assembly of the mitotic spindle and ensure that replicated chromosomes attach to the spindle. In many organisms they trigger chromosome condensation, nuclear envelope breakdown, actin cytoskeleton rearrangement and the reorganization of Golgi apparatus and Endoplasmic reticulum. In each of these events M-Cdks phosphorylates the respective regulatory proteins.

Eg 1: Breaking down nuclear envelope: Phosphorylation of the polymerized lamin filament (That makes up nuclear envelope and makes it rigid) by M-Cdk results in their depolymerization which is the first step in breakdown of nuclear envelop.

Eg 2. Condensation of chromosomes in Xenopus, requires a complex of fine proteins. Phosphorylation of this condensing by M-Cdk promotes coiling of DNA molecules.

Eg 3. Spindle formation requires assembly of microtubules. Phosphorylation of large number of proteins that regulate microtubule behavior is by M-Cdk.

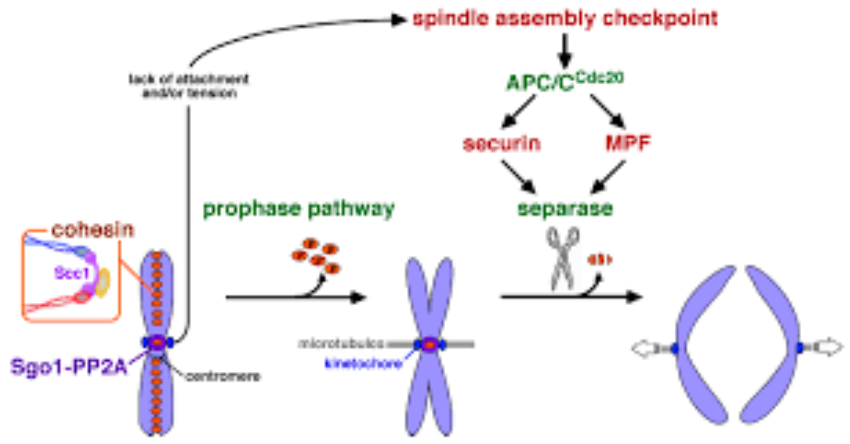
**5) Sister chromatid separation is triggered by Proteolysis:**

After M-Cdk has triggered the complex rearrangements that occur in early mitosis, the cell cycle reaches a stage where sister chromatids separation has to take place during the transition called Metaphase – to- Anaphase transition. This is by Anaphase promoting complex (APC).

The sister chromatids are bound tightly by complex of proteins- The Cohesion complex that deposits along the chromosomes in S phase. anaphase must involve disruption of this cohesion such that they move to opposite poles of the spindle. This process is initiated by a remarkable cascade of signaling events. It requires action of APC enzyme complex whose target is the protein Securin. Securin binds to protease Separase and prevents cleavage of Cohesion complex binding the sister Chromatids.

Events of APC activation require protein Cdc 20. As such Cdc20 concentration increases, as cell approaches mitosis. Thus phosphorylation of APC binds Cdc 20 to APC. This Cdc20-APC complex causes proteolysis of Securin, releasing Separase which helps in separation of sister chromatids (Figure 4.14).

Figure 4.14: Separation of sister chromatids triggered by proteolysis



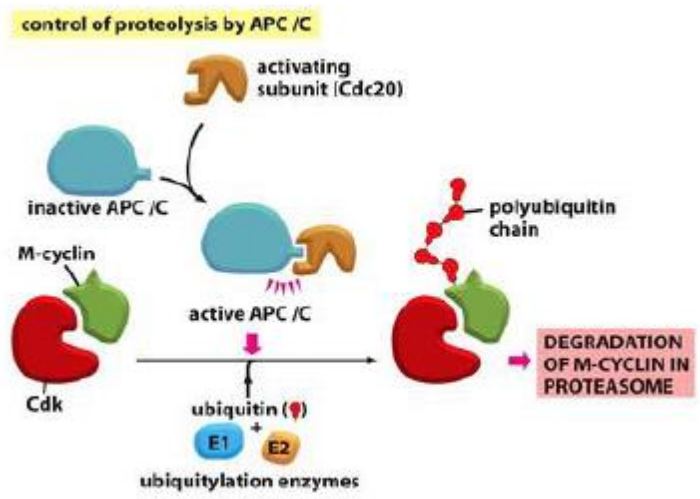

**6) The spindle Attachment checkpoint:**

In most cells, this check point operates to ensure that all chromosomes are properly attached to the spindle before the sister chromatids separate. The sensor mechanism monitors the state of the Kinetochore, to which microtubules or spindle attaches. Single kinetochores recruit Mad2 and allow their binding. Binding of spindle releases Mad2, and if any kinetochore is still left withMad2 it inhibits Cdc20 –APC activity and destruction of Securin.

**7) Exit from Mitosis requires the inactivation of M-Cdk:**

Mitosis exit must involve disassembly of spindle, decondensation of chromosomes and reformation of nuclear envelop. These are the reverse of events triggered by M-Cdk. Hence inactivation of M-Cdk has to take place which is mainly by Ubiquitin- dependent proteolysis (figure 4.15). Ubiquitylation of cyclins is usually triggered by Cdc20 – APC complex that promotes destruction of Securin, promotion from metaphase to Anaphase and also inactivation of M-Cdk.

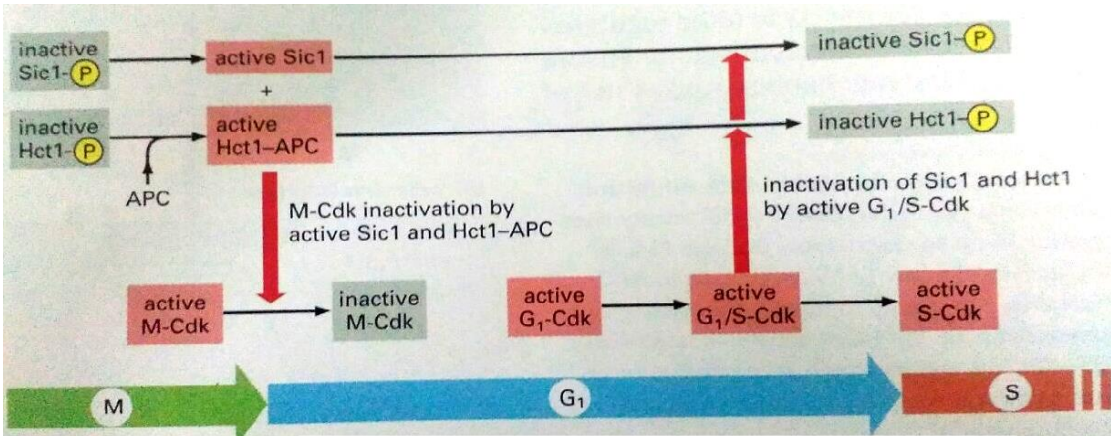
Figure 4.15: Degradation of M- cyclins




**8) M-Cdk activity inhibition promotes cells into $G_1$ phase:**

Cells adopt several mechanisms to ensure that Cdk reactivation is prevented after mitosis. One mechanism makes use of another APC-activating protein called Hct 1, a close relative of Cdc 20. The Hct 1- APC complex is inhibited by M-Cdk, which directly phosphorylates Hct1. As a result of this, Hct 1-APC activity increases in late mitosis after the Cdc20-APC complex has initiated destruction of M-Cyclins. Therefore M-cyclin destruction continues after mitosis, althoughCdc-20- APC activity has declined, Hct 1-APC activity is high (figure 4.16).

Figure 4.16: The control of G1 progression by Cdk activity



Second mechanism is that which suppresses Cdk activity in $G_1$ phase, which depends on increased production of CKI s. The Cdk inhibitory protein, like Hct1, Sic 1 is inhibited by M-Cdk which phosphorylates Sic 1 during mitosis. M-Cdk also phosphorylates and inhibits a gene regulatory protein required for Sic1 synthesis. This Sic1 and M-Cdk, like Hct 1 and M-Cdk mutually inhibit each other. Late mitosis triggers rapid accumulation of Sic 1 protein, and the CKI helps to ensure that M-Cdk activity is stably inhibited.

Then How do cells enter S Phase?? Hct 1- APC activation, CKI accumulation and decrease cyclin production ensure cell progression into $G_1$ phase and Cdk activity is suppressed.

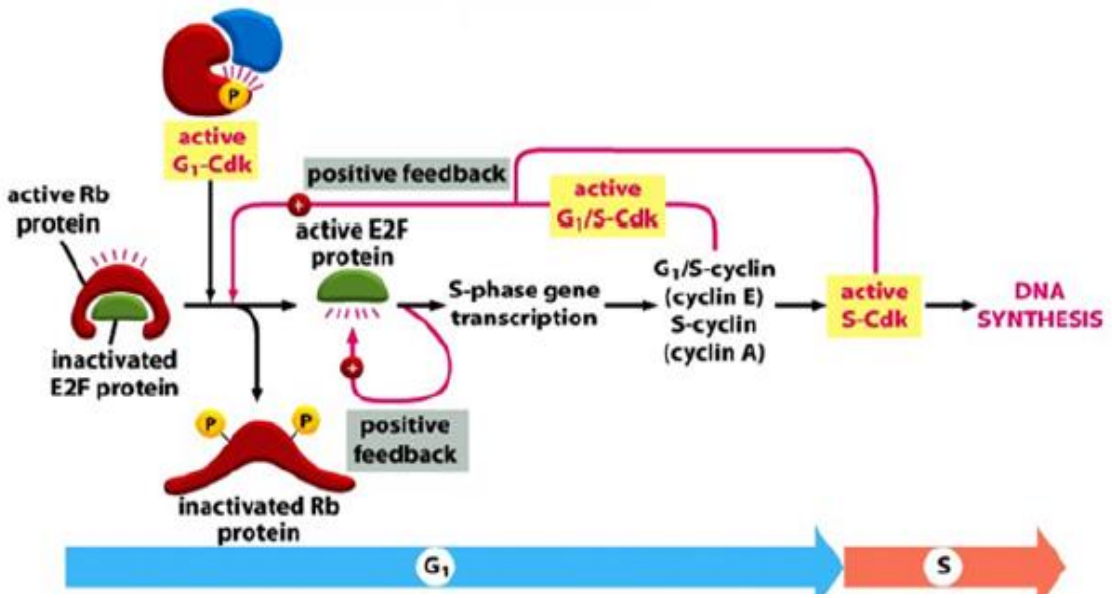
Cells start accumulation of $G_1$ – cyclins which is stimulated by the extracellular signals that promote cell proliferation. Accumulation of $G_1$ cyclins leads to an increase in $G_1$- Cdk activity. In yeast, $G_1$- Cdk activity triggers transcription of $G_1$/S – cyclin genes leading to increased synthesis of $G_1$/S – Cdl complexes. This initiates events that commit to enter S-phase. it stimulates transcription of S-cyclin genes, resulting in synthesis of S-cyclins and formation of S-Cdk complexes. These complexes are inhibited by Sic 1, but $G_1$/S- Cdk phosphorylates and inactivates sic 1, thereby counting S-Cdk activation. $G_1$/S- Cdk and S-Cdk also phosphorylates and inactivates Hct 1 – APC. Thus the same feedback loops that trigger rapid M-Cdk inactivation in late mitosis now work in reverse at the end of $G_1$ phase to ensure the rapid and complete activation of S-Cdk activity (figure 4.16).

**In mammalian cells:** The transition from $G_1$ to S phase in mammals is mediated by a gene regulatory protein called E2F. E2F binds to specific DNA sequences in the promoter regions of genes that encode for S-phase entry including $G_1$/S cyclins and S- cyclin. Function of E2F is controlled by Retinoblastoma protein (Rb), an inhibitor of cell cycle progression.

In $G_1$ phase, Rb binds to E2F blocking the transcription of S-phase genes. Accumulation of active $G_1$- Cdk due to stimulus by external factors, it phosphorylates Rb, reducing affinity for E2F. E2F release activates gene expression of S-phase genes (Figure 4.17).

Loss of Rb gene leads to excessive cell proliferation in the immature retina (eye cancer) known as Retinoblastoma.

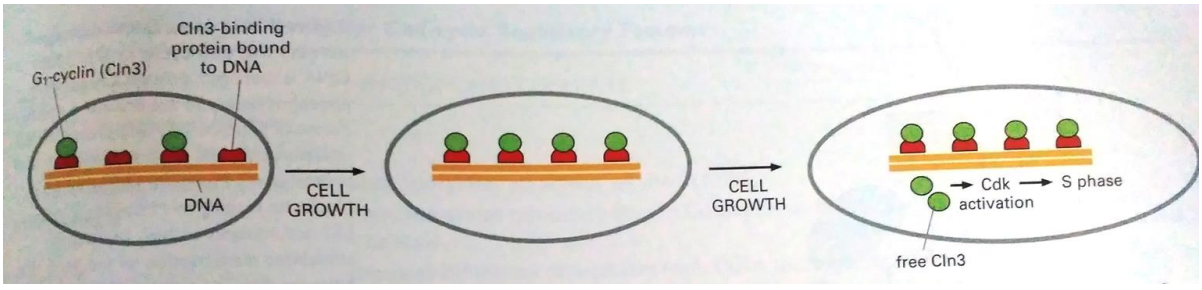
Figure 4.17: Mechanism controlling S-phase initiation in animalcells.




**Cell cycle progression and cell growth:**

Cell growth depends on nutrients and growth signals from environment. The length of time depends on nutrients availability. This coordination of cell cycle is based on nutrient availability and is attributed to the total amount of $G_1$ cyclins called Cln3. Increase in Cln3 stimulates cell division, even if cells are small. As cells grow, the total number of Cln3 molecules increase in parallel with total cell protein. When cells are small a Cln3 binding protein inactivates its activity. As cell grows and reaches its threshold size, the number of Cln3 increase along with Cln3 binding protein. When cell exceeds its size, free Cln3 exist, which can bind to Cdk and initiate a new cell cycle (figure 4.18).

Figure 4.18: Regulation of cell cycle progression and cell growth by Cln3

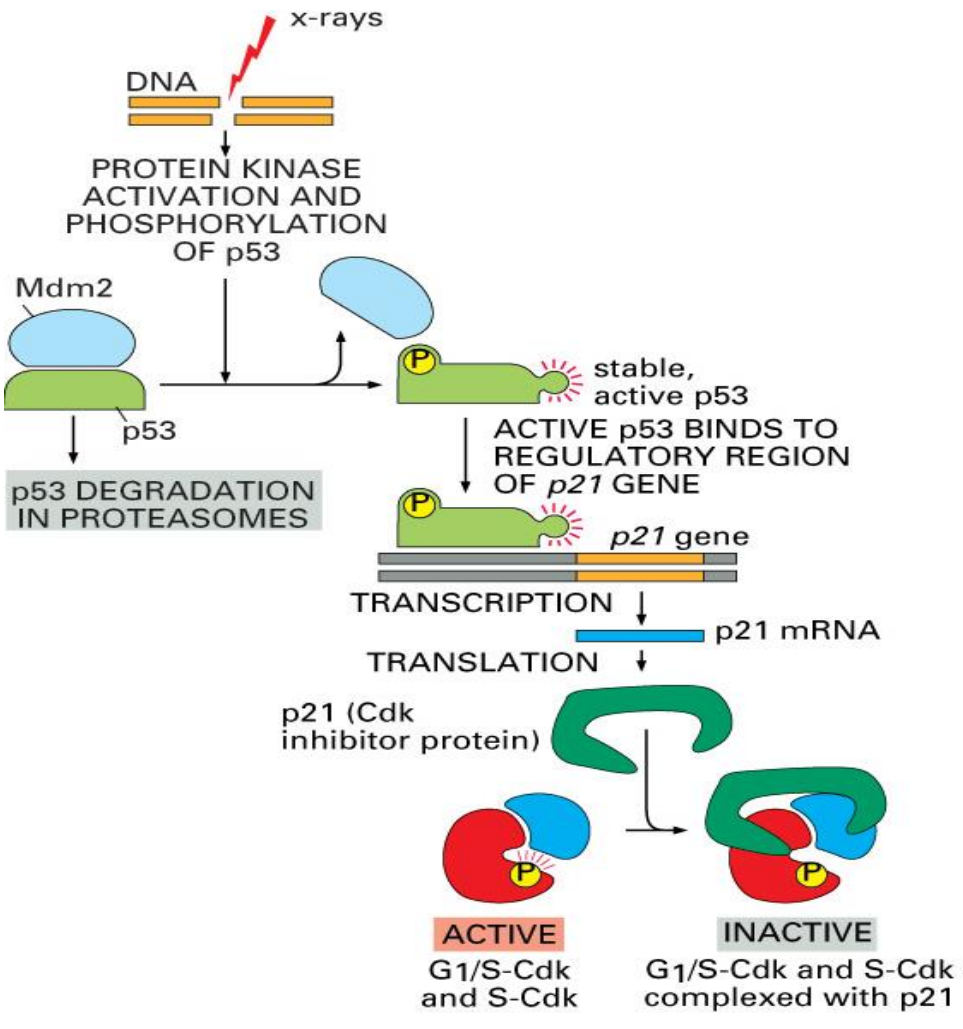


**Blocking of Cell cycle progression by damaged DNA and p53:**

This is DNA damage check point. There are 2 such check points – one at late $G_1$ phase which prevents entry into S phase and the other at late $G_2$, which prevents entry into mitosis. A $G_1$ check point, DNA damage leads to activation of the gene regulatory protein p53, which stimulates transcription of many genes, of which CKI protein called p21, that binds to $G_1$/S – Cdk and S-Cdk and inhibits cell activities thereby blocking entry into S phase.

p53 operates by indirect mechanism. In undamaged cells, p53 is highly unstable and is present in low concentrations. It interacts with Mdm2, that acts as a Ubiquitin ligase and targets p53 for destruction by proteosomes. DNA damage activates protein kinase which phosphorylates p53 that reduces binding to Mdm 2. Increase in p53, stimulates gene transcription (figure 4.19) of p21 that prevents entry into S phase.

Figure 4.19: Blocking cell cycle by damaged DNA and p53



Summary of cell cycle and its control system: