PHASES OF CELL CYCLE

It consists of 2 major activities.

- **INTER PHASE**
  - **G_1 (pre-synthetic phase)**
  - **S (DNA synthesis)**
  - **G_2 (pre-mitotic phase)**

- **CELL DIVISION (MITOTIC PHASE)**

- **a) Interphase-** During this phase the cell grows, accumulating nutrients needed for mitosis and duplicating its DNA.
- **b) Mitosis (M)-phase-** During which the cell splits itself into two distinct cells.
- The duration of the cell cycle varies from hours to years. A typical human cell has duration of 90h.

INTERPHASE

- **A)INTERPHASE:** It is the longest phase. In a typical human cell, out of the 90h, interphase lasts for 89h.
- **CHARACTERS OF INTERPHASE:** It is the resting phase of the cell. Resting refers to the rest from division. But, the cells in the interphase are metabolically active. The metabolic activities are high in this phase. The cell grows during phase. During this phase mRNA and rRNA are synthesized. The chromosomes duplicates into two chromatids. The centrioles duplicates into two. Thus two centrioles are formed. The centrospheres of centrioles, microtubules arise. These microtubules form asters.
-
- **STAGES OF INTERPHASE:** Interphase consists of 3 sub-stages. They are

1. **G_1 phase**
2. **S phase**
3. **G_2 phase**

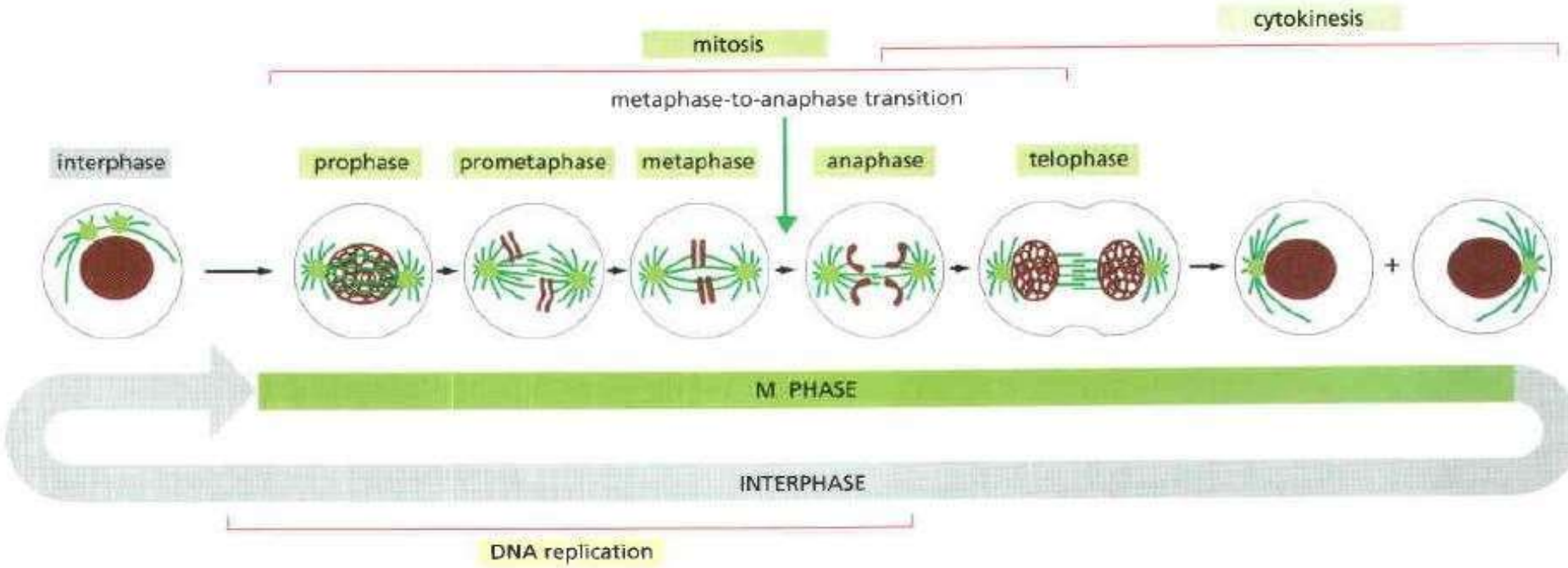
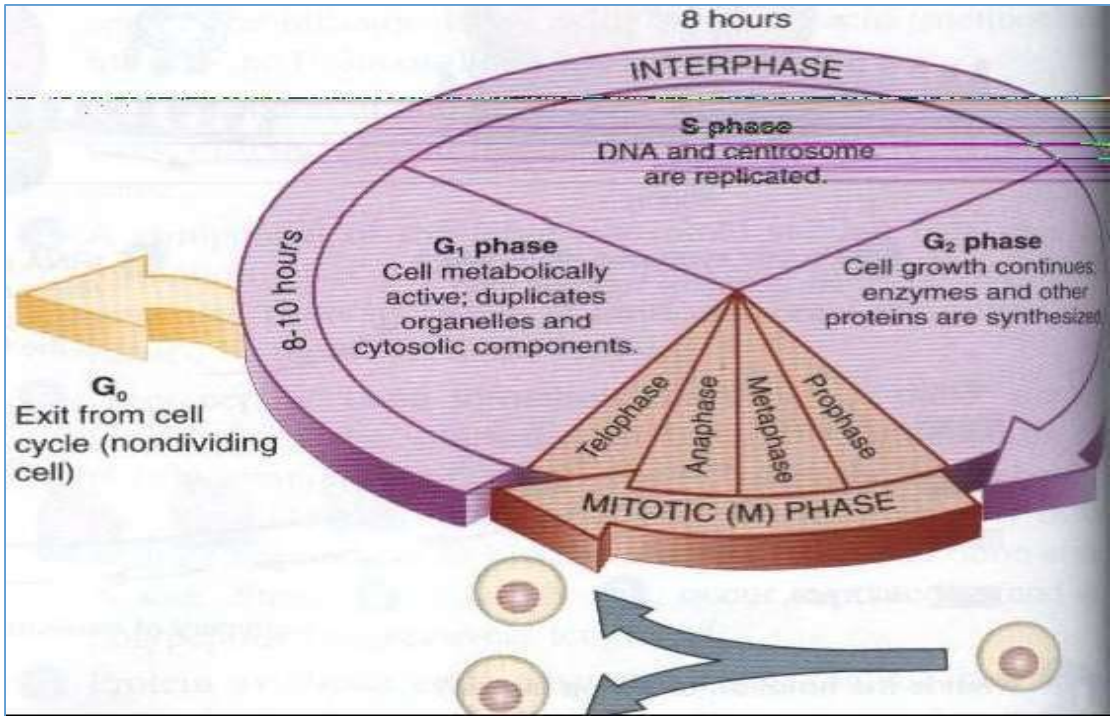


Figure 17–3 The events of eucaryotic cell division as seen under a microscope. The easily visible processes of nuclear division (mitosis) and cell division (cytokinesis), collectively called M phase, typically occupy only a small fraction of the cell cycle. The other, much longer, part of the cycle is known as interphase, which includes S phase and the gap phases (discussed in text). The five stages of mitosis are shown: an abrupt change in the biochemical state of the cell occurs at the transition from metaphase to anaphase. A cell can pause in metaphase before this transition point, but once it passes this point, the cell carries on to the end of mitosis and through cytokinesis into interphase.



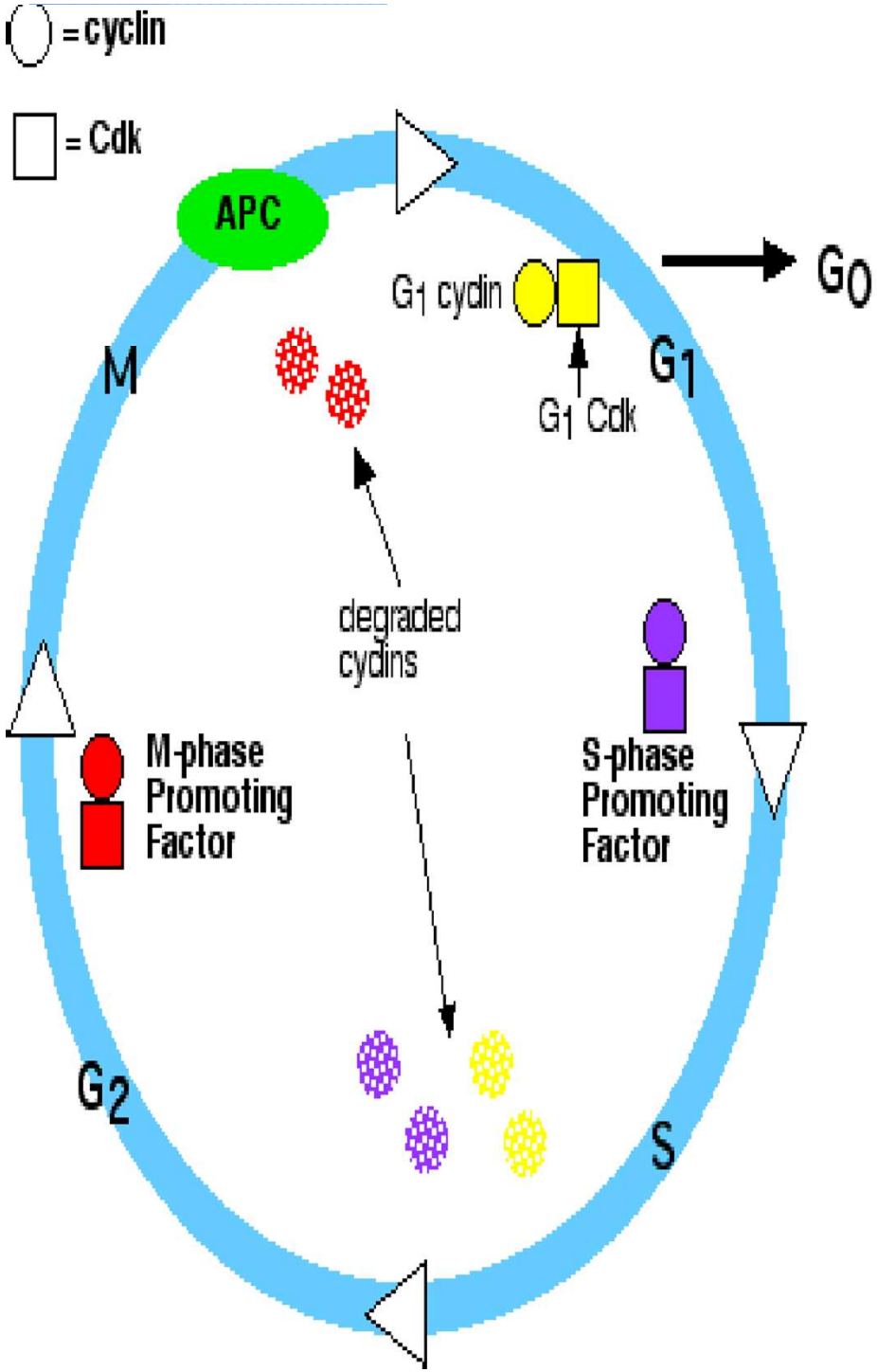
G_0 PHASE

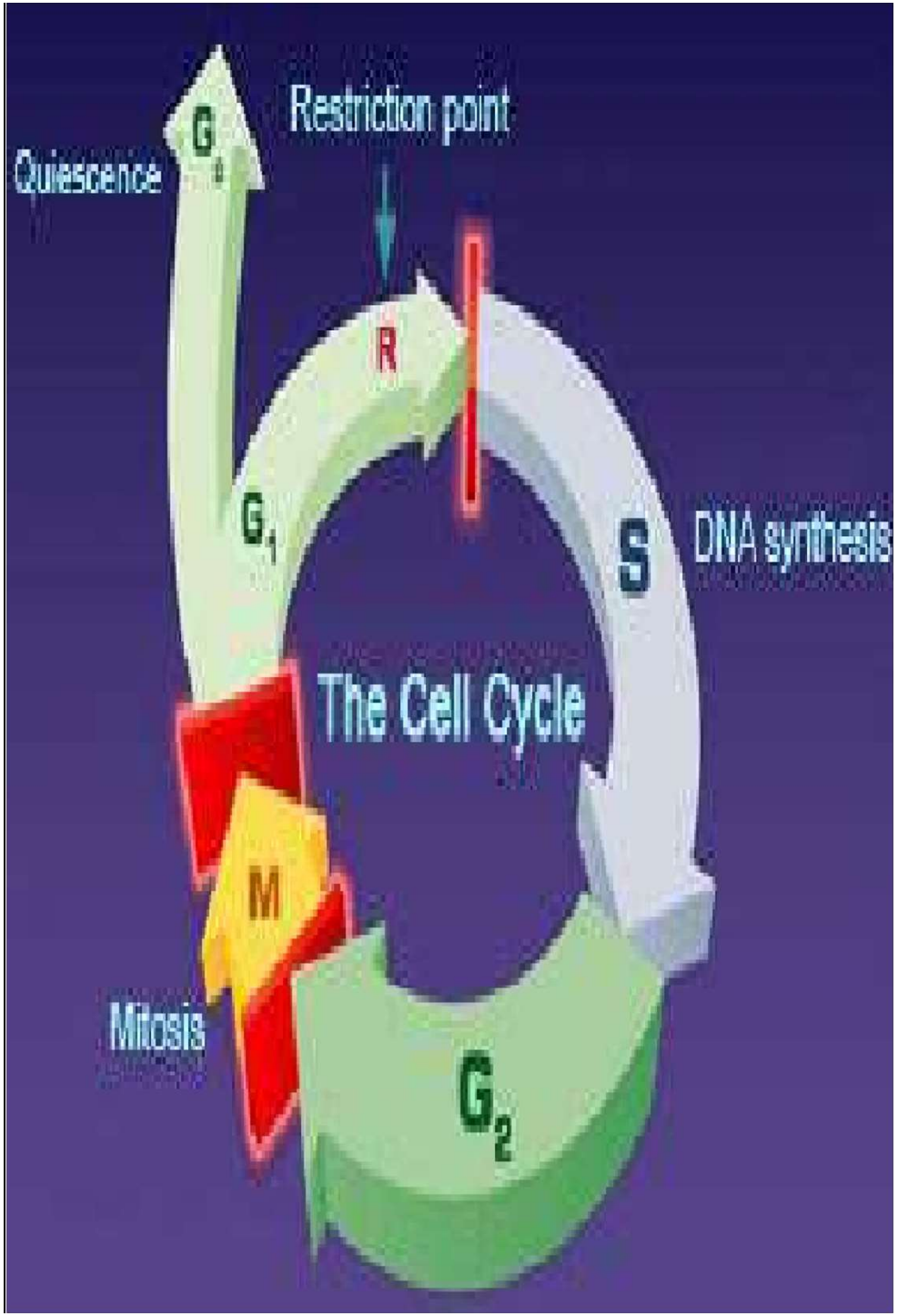
- It is the resting phase.
- In these cells cyclin D is in decreased concentration.
- Rb protein is in hypo-phosphorylated (active form).
- Hence, holds the cell cycle at check point 1 by inhibiting the expression of several transcription proteins(E2F) that codes cyclins A and E necessary for cycle progression.
- Growth factor stimulation takes the G_0 cells to G_1 phase.
- In interphase the cell prepares itself to cycle. The term post-mitotic is sometimes used to refer both quiescent and senescent cells.
- Non-proliferative cells in eukaryotes generally enter the quiescent Go state from G_1 and may remain quiescent for longer period of time or indefinitely (e.g.cardiac cells and neurons). In multicellular eukaryotes, cells enter the Go phase from the G_1 phase and stop dividing. Some cells enter the Go phase semi-permanently, e.g.some liver and kidney cells.

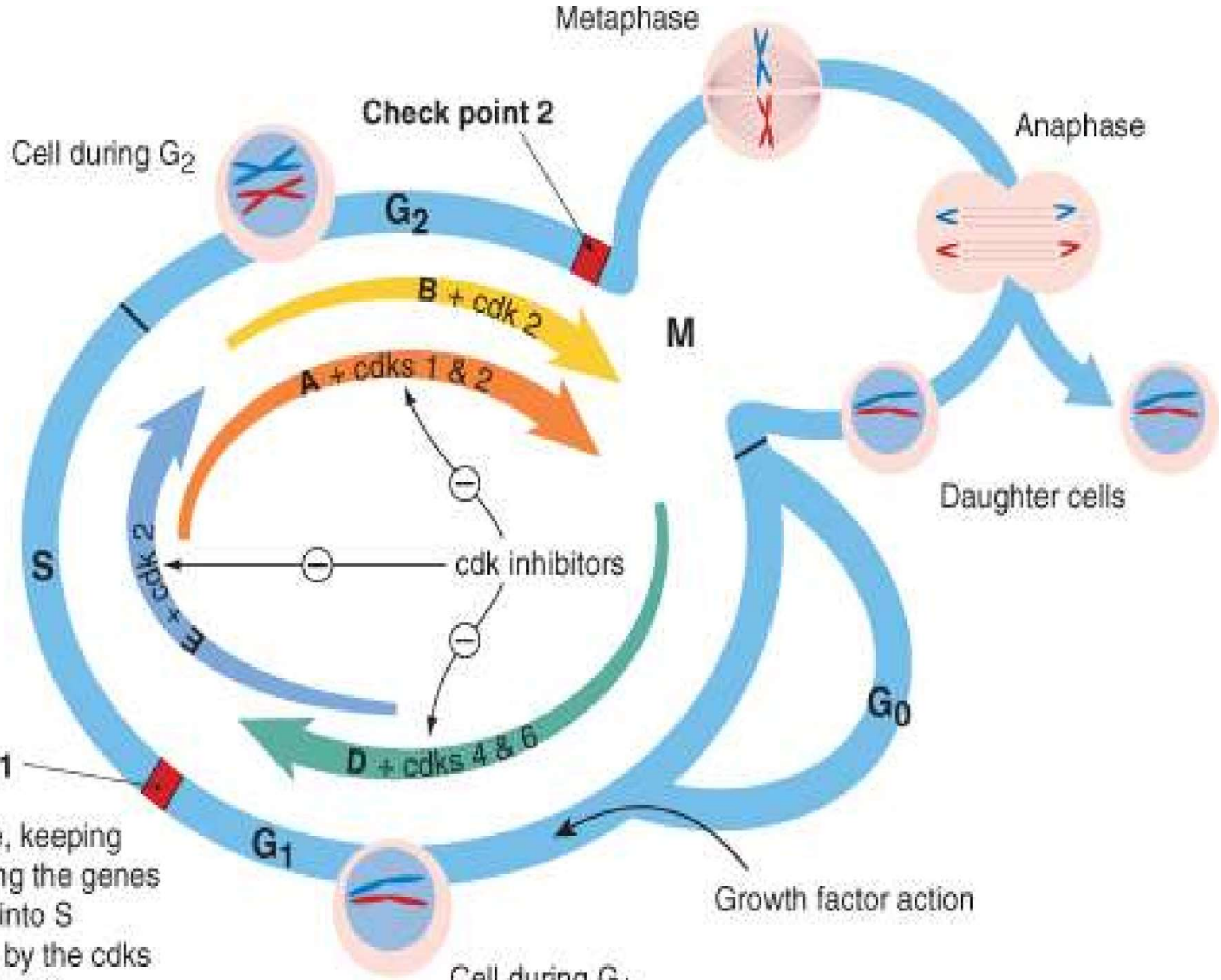
G_1 PHASE

- G stands for gap. It is the first phase within the interphase.
- It is also called the growth phase. This phase is the gap period between a mitotic phase and the S phase of the cycle.
- Cell is preparing for S phase.
- This period starts immediately after division. The daughter cells grow and increase in size during this phase.
- It is a longer phase. It lasts for even years. The nerve cells remain permanently in G_1 phase. Generally, this stage lasts for 25 to 50% of the total interphase.
- During this phase 20 amino acids are formed, from which millions of proteins and enzymes are formed, which are required in S phase. During this phase mRNA, rRNA and tRNAs are formed. During this phase new cell organelles are formed.
- Concentration of cyclin D increases, resulting in phosphorylation and activation of necessary transcription proteins resulting in synthesis of DNA polymerase DHFR etc which is required for DNA replication.
- Formation of cyclin E complex is necessary for the transition from G_1 to S phase (check point 1)

- G1 phase consists of four sub-phases:
  - **Competence (g1a)**
  - **Entry (g1b)**
  - **Progression (g1c)**
  - **Assembly (g1d)**
- These sub-phases may be affected by limiting growth factors, nutrient supply, temperature, and additional inhibiting factors.
- A rapidly dividing human cell which divides every 24h spends 9h in G_1 phase. The DNA in a G_1 diploid eukaryotic cell is 2n, meaning there are two sets of chromosomes present in the cell. The genetic material exists as chromatin.
- There is a restriction point present at the end of G_1 phase.
- Signals from extracellular growth factors are transducer in a typical manner. Growth factors bind to receptors on the surface. Accumulation of cyclin D is essential. Cyclins are a family of proteins that control the progression of cells through the cell cycle by activating cyclin-dependent kinases (Cdk) enzymes. Cyclin D acts as a mitogenic signal sensor.


= cyclin
= Cdk
APC
G1 cyclin
G1 Cdk
G0
M
G1
degraded cyclins
M-phase Promoting Factor
S-phase Promoting Factor
G2
S


Quiescence
G0
Restriction point
R
G1
S
DNA synthesis
The Cell Cycle
M
Mitosis
G2



Rb acts as a brake here, keeping the cell in G_1 by inhibiting the genes necessary for the entry into S phase; phosphorylation by the cdks releases the brake. The p53 protein stops the cycle here if there has been DNA damage

S PHASE

- Cyclin E/cdk and cyclin A/cdk regulate the processes in phase S.
- By phosphorylating and activating proteins and enzymes that are involved in DNA synthesis.
- S stands for synthesis. During this phase DNA synthesis occurs. The DNA molecule duplicates. All the chromosomes have been replicated. This period lasts for 35 to 40% of interphase.
- During this phase, synthesis is completed as quickly as possible due to the exposed base pairs may be destroyed by the external proteins (drugs) or any mutagens (such as nicotine).
- Cyclins, when bound with the dependent kinases such as Cdk1 proteins form the maturation- promoting factor (MPF). MPFs activate other proteins through phosphorylation. These phosphorylated proteins, in turn, are responsible for specific events during cell division such as microtubule formation and chromatin remodeling.

G_2 PHASE

- Pre-mitotic phase.
- The G_2 phase is the gap period between S-phase and mitotic (M) phase of a cell cycle. It is the second growth phase. It is a period of rapid cell growth and protein synthesis which the cell ready itself for mitosis.
- The nucleus increases in volume. Metabolic activities essential for cell division, occur during this phase. mRNA, tRNA and rRNA synthesis also occur. It is not a necessary part of the cell cycle.
- This phase has double the number of chromosomes.
- All the other cellular components are duplicated for the 2 daughter cells.
- Cyclin A/cdk and cyclin B/cdk complexes are active which are necessary for the cell to enter into M phase (check point 2).

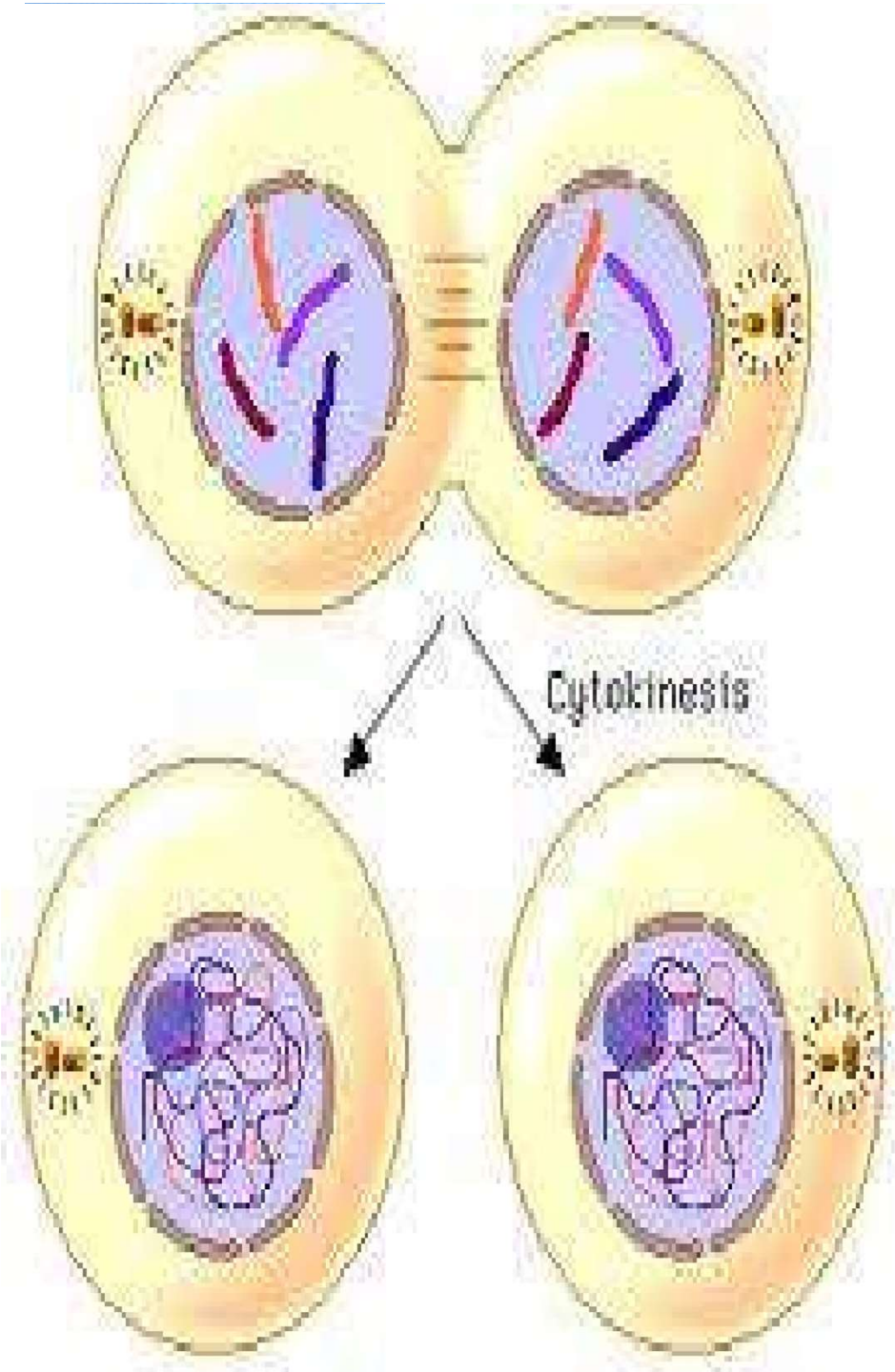
DNA DAMAGE CHECKPOINTS

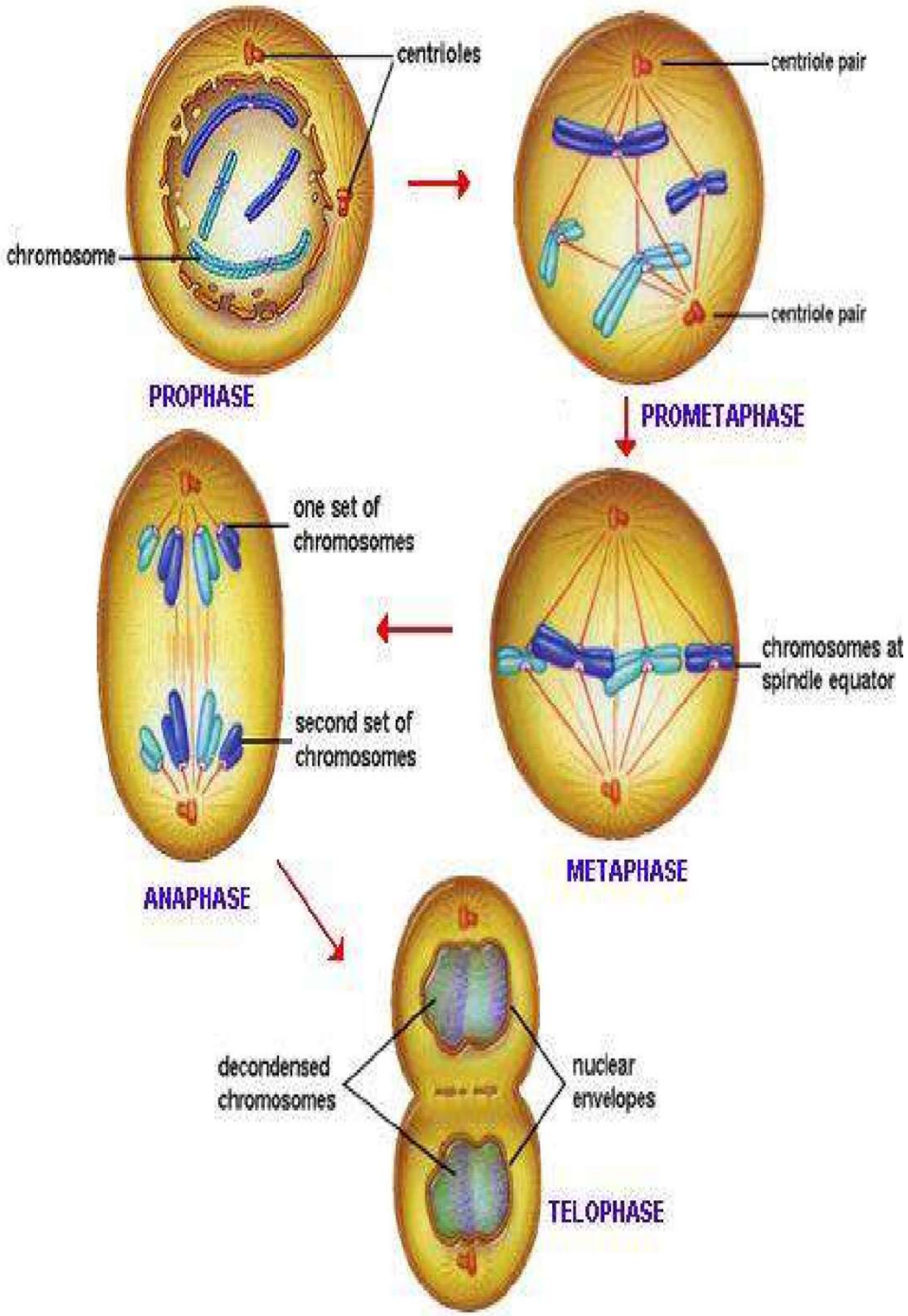
- These sense DNA damage both before the cell enters S phase (a G_1 checkpoint) as well as after S phase (a G_2 checkpoint).
- Damage to DNA before the cell enters S phase inhibits the action of Cdk2 thus stopping the progression of the cell cycle until the damage can be repaired. If the damage is so severe, that it cannot be repaired, then the cell destructs by apoptosis.
- Damage (UV radiation, oxidative stress, etc) to DNA after S phase (the G_2 checkpoint) inhibits the action of Cdk1 thus preventing the cell from proceeding from G_2 to mitosis.
- In the S phase if DNA replication stops at any point on the DNA, the progress through the cell cycle is halted until the problem is solved.

M PHASE (MITOSIS)

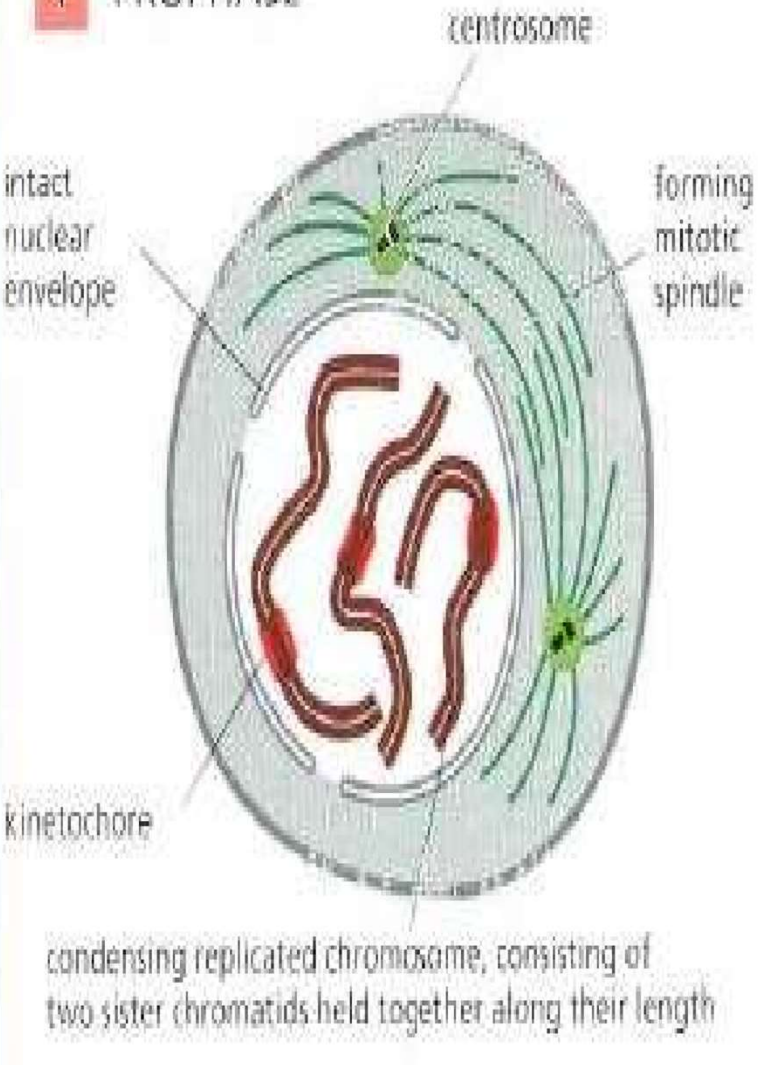
- G_2 cells are divided into two daughter cells which may enter the cycle again at G_1 phase or come out of the cycle to G_0 phase.
- Mitosis is the distribution of the two sets of chromosomes into two separate and equal nuclei.
- This is the division phase. During this phase the cell divides. This phase has a short duration. A typical human cell cycle has duration of 90h. Of these the M phase has duration of 45 to 60min. This phase has two sub-phases called karyokinesis and cytokinesis.
- Karyokinesis refers to the cell division of nucleus into two daughter nuclei. It has 4 sub-stages, namely prophase, metaphase, anaphase and telophase.
- Cytokinesis refers to the cell division of the cytoplasm resulting in two daughter cells.

1) **Pro phase- mitotic spindle formation**
2) **Meta phase- metaphase plate**
3) **Ana phase- mitotic apparatus**
4) **Telo phase- cytokinesis**

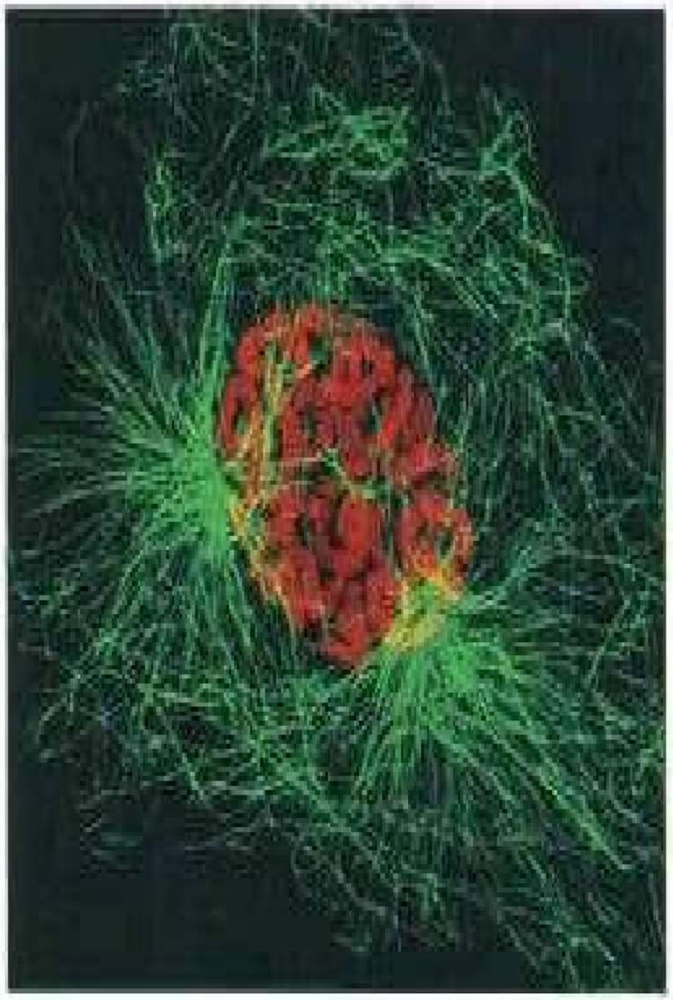

Cytokinesis


centrioles
chromosome
PROPHASE
centriole pair
centriole pair
PROMETAPHASE
chromosomes at
spindle equator
METAPHASE
one set of
chromosomes
second set of
chromosomes
ANAPHASE
decondensed
chromosomes
nuclear
envelopes
TELOPHASE

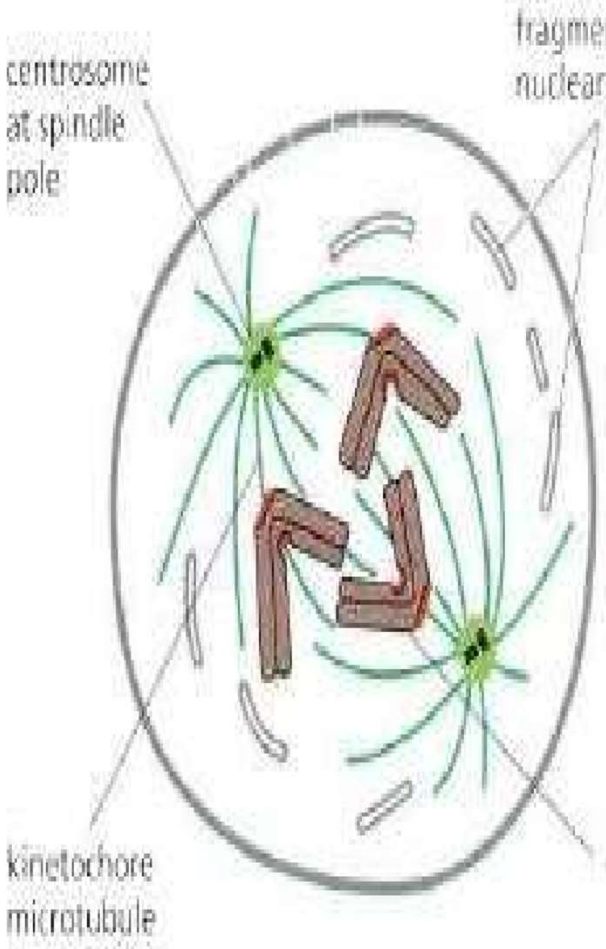
1 PROPHASE



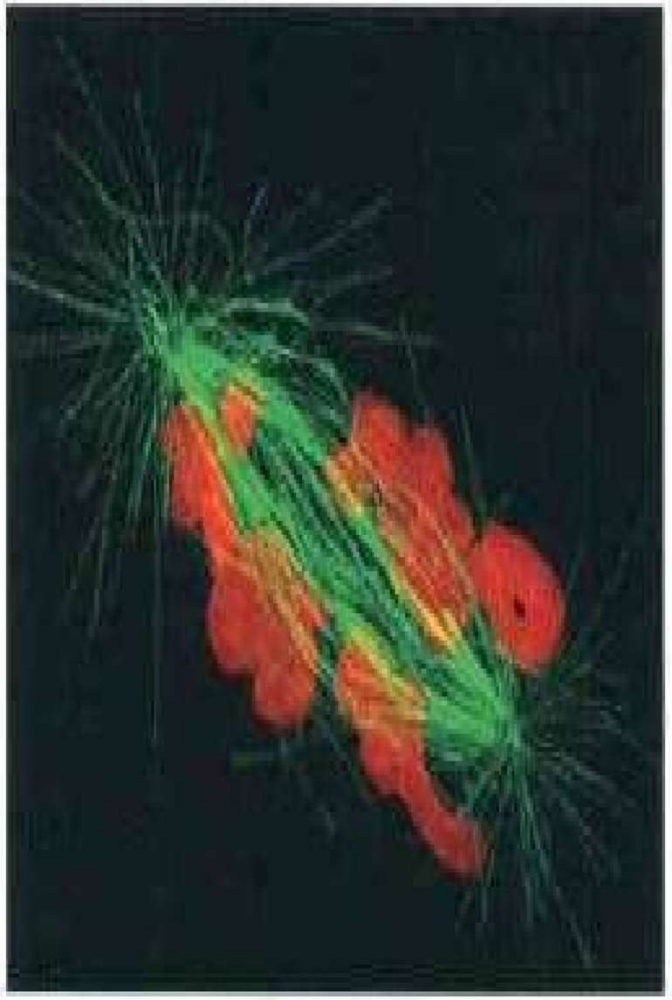
At prophase, the replicated chromosomes, each consisting of two closely associated sister chromatids, condense. Outside the nucleus, the mitotic spindle assembles between the two centrosomes, which have replicated and moved apart. For simplicity, only three chromosomes are shown. In diploid cells, there would be two copies of each chromosome present. In the photomicrograph, chromosomes are stained *orange* and microtubules are *green*.



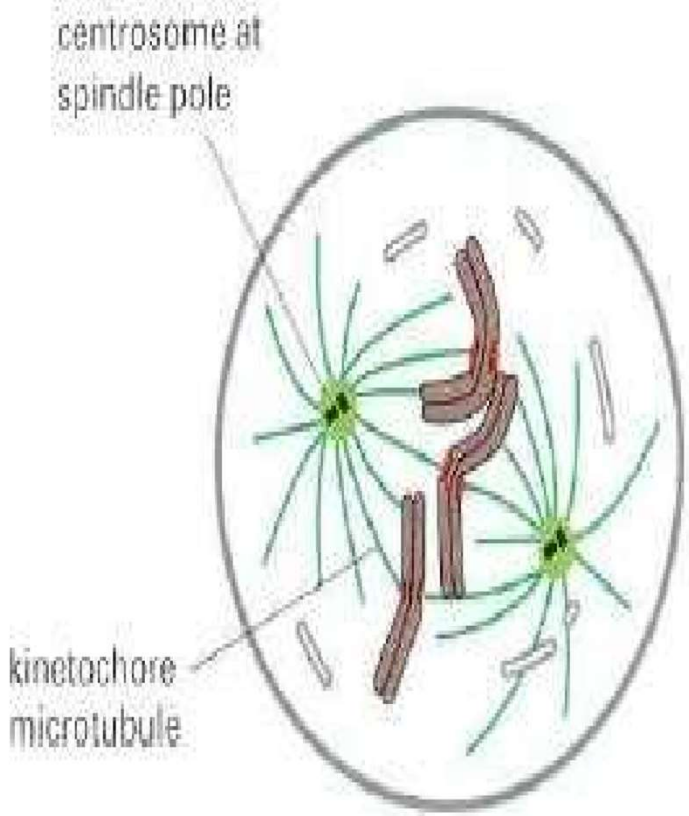
2 PROMETAPHASE



Prometaphase starts abruptly with the breakdown of the nuclear envelope. Chromosomes can now attach to spindle microtubules via their kinetochores and undergo active movement.

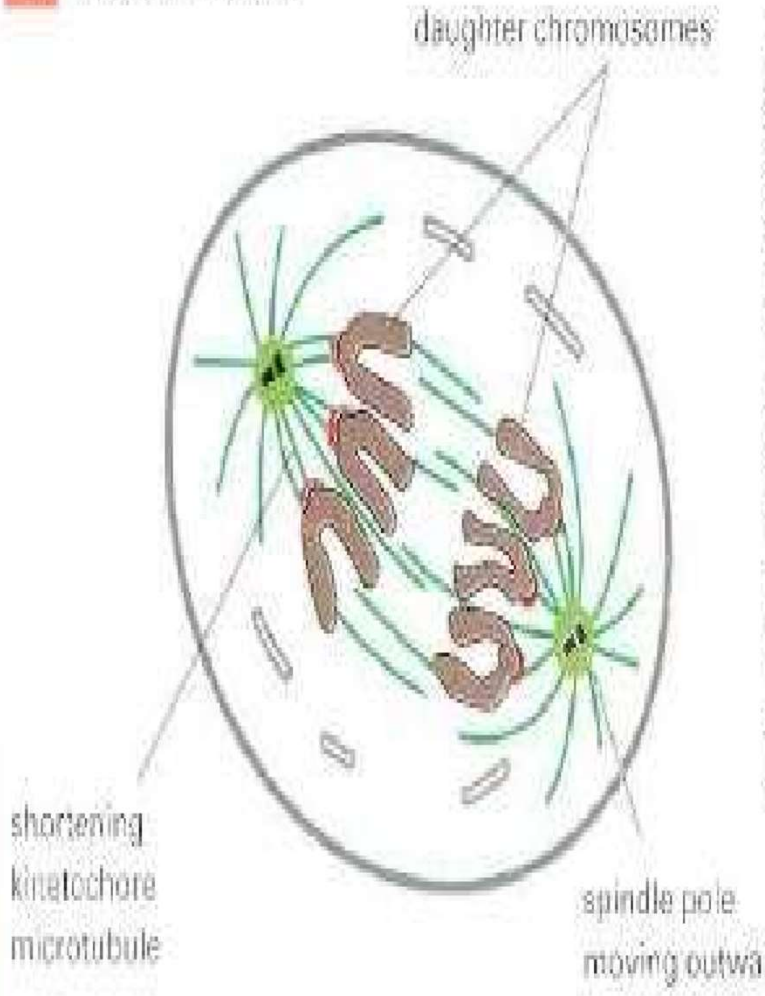


3 METAPHASE

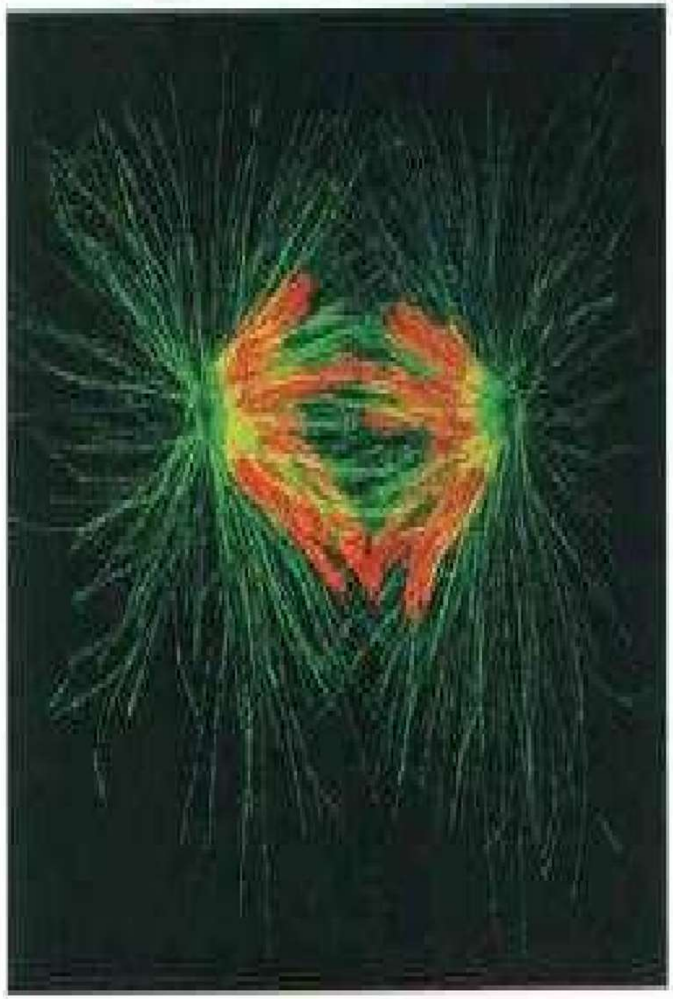


At metaphase, the chromosomes are aligned at the equator of the spindle, midway between the spindle poles. The kinetochore microtubules attach sister chromatids to opposite poles of the spindle.

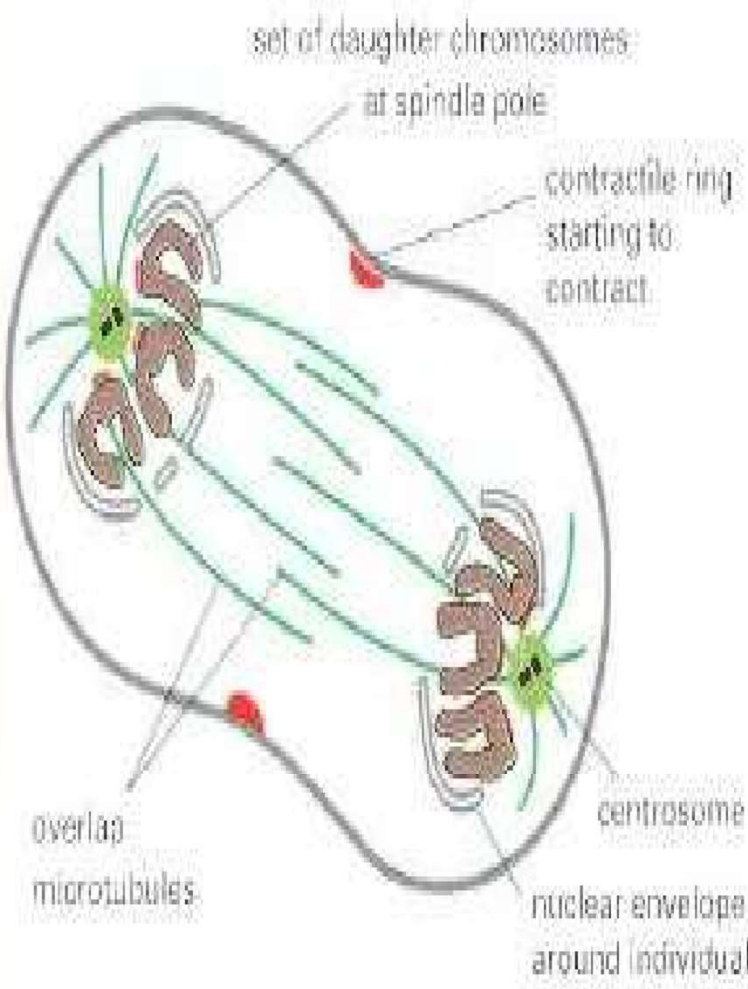
4 ANAPHASE



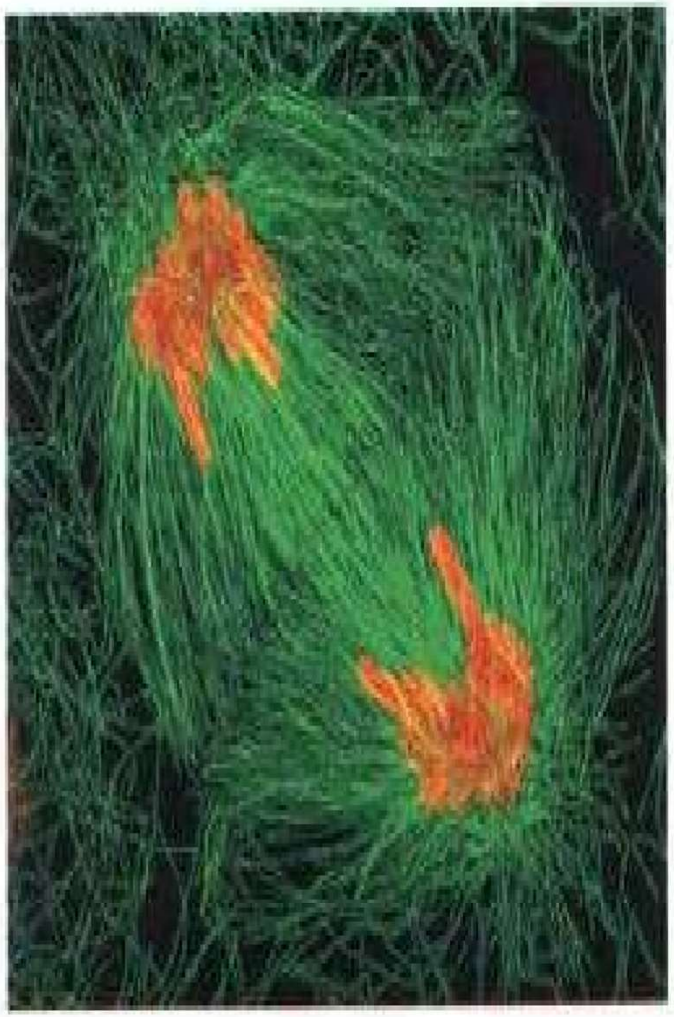
At anaphase, the sister chromatids synchronously separate to form two daughter chromosomes, and each is pulled slowly toward the spindle pole it faces. The kinetochore microtubules get shorter, and the spindle poles also move apart; both processes contribute to chromosome segregation.



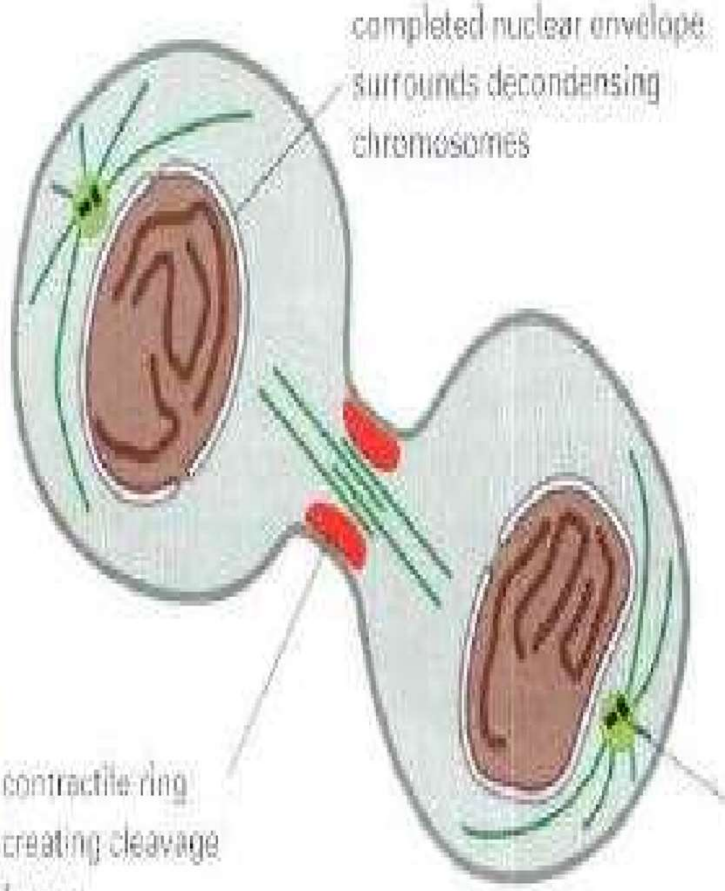
5 TELOPHASE



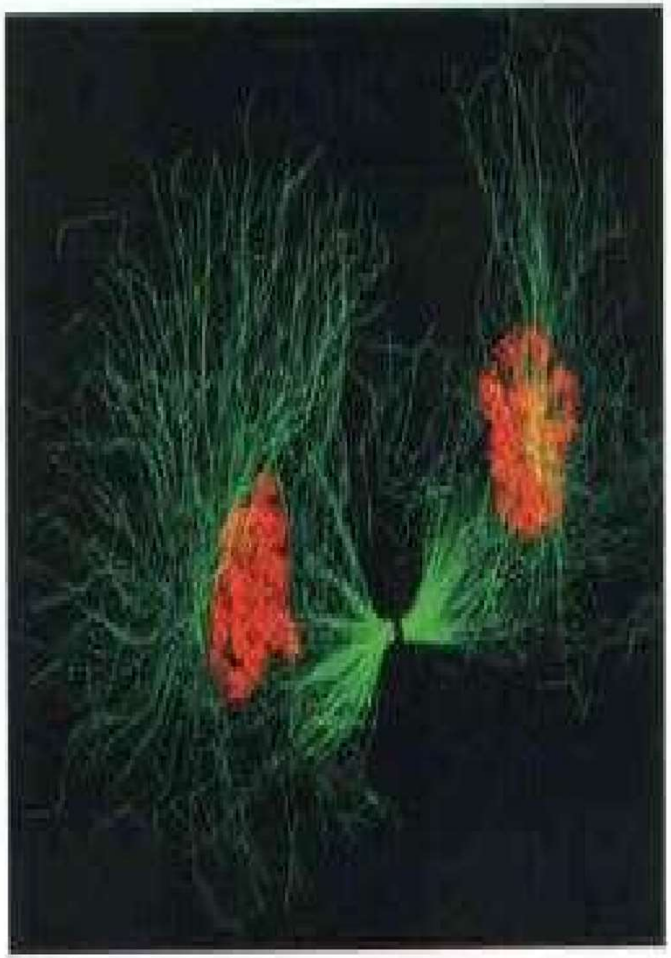
During telophase, the two sets of daughter chromosomes arrive at the poles of the spindle and decondense. A new nuclear envelope reassembles around each set, completing the formation of two nuclei and marking the end of mitosis. The division of the cytoplasm begins with contraction of the contractile ring.



6 CYTOKINESIS



During cytokinesis, the cytoplasm is divided in two by a contractile ring of actin and myosin filaments, which pinches the cell in two to create two daughters, each with one nucleus.



(Micrographs courtesy of Julie Canman and Ted Salmon.)

CELL CYCLE CHECKPOINTS (RESTRICTION POINTS)

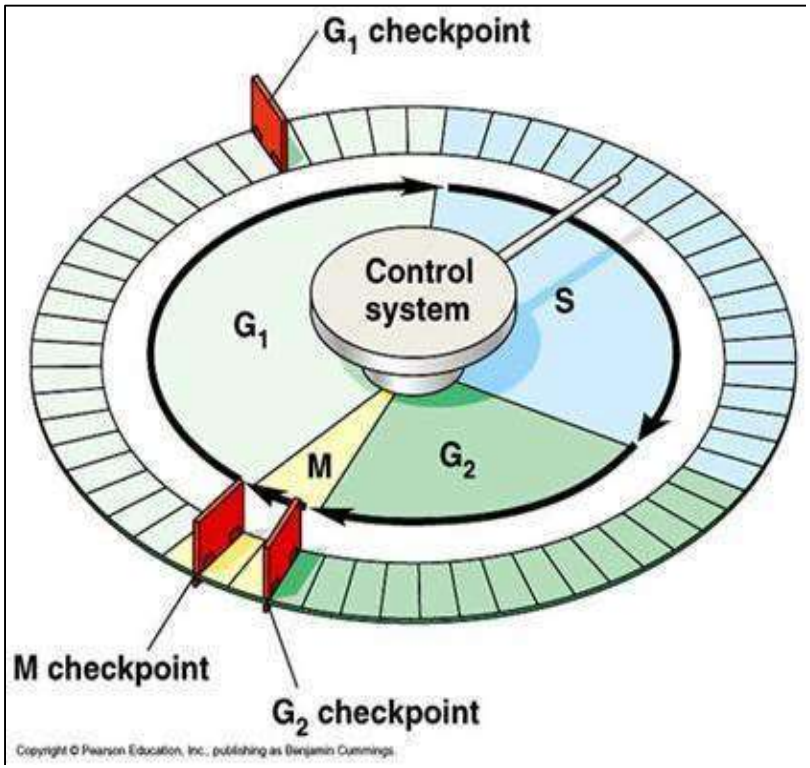
- These are the cell cycle control mechanisms in eukaryotic cells. These checkpoints verify whether the processes at each phase of cell cycle have been accurately completed before progression into the next phase. There are three main checkpoints that control the cell cycle in eukaryotic cells.

- They are -

1. G_1 checkpoint (G_1 restriction point)

2. G_2 checkpoint

3. Metaphase checkpoint



CELL CYCLE CHECKPOINTS (RESTRICTION POINTS)

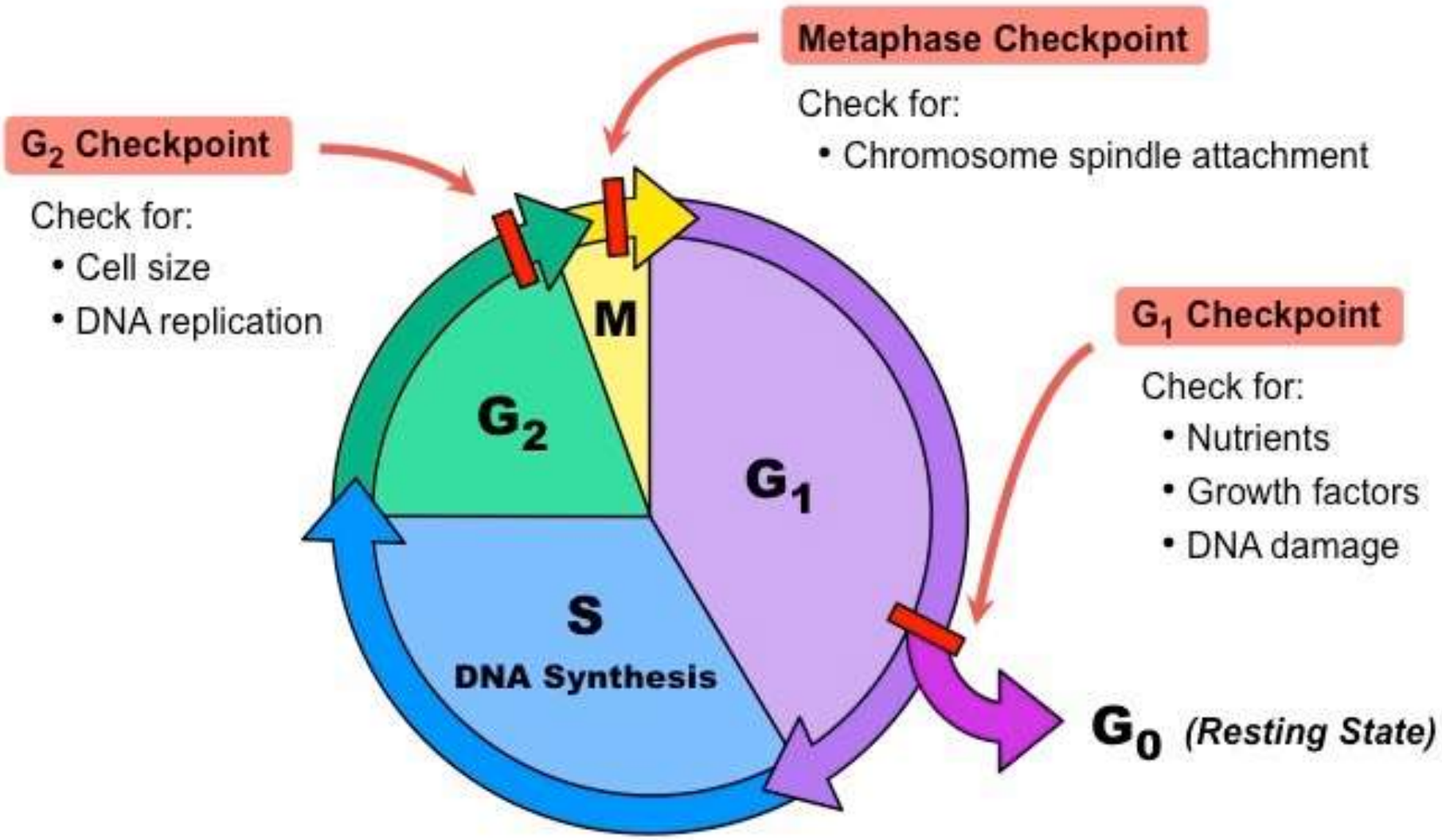
- They are -

1.G_1 / G1-S checkpoint

2.G_2 or G2-M checkpoint

3.Metaphase checkpoint/ anaphase Checkpoint/

Spindle assembly checkpoint


Metaphase Checkpoint
Check for:
• Chromosome spindle attachment
G2 Checkpoint
Check for:
• Cell size
• DNA replication
G1 Checkpoint
Check for:
• Nutrients
• Growth factors
• DNA damage
M
G2
G1
S
DNA Synthesis
G0 (Resting State)

G_1 checkpoint (G_1 restriction point)

- Checks for
  1. cell size
  2. Nutrients
  3. growth factors
  4. DNA damage

•The G_1 checkpoint determines whether all conditions are favorable for cell division to proceed or not. Such as damage to DNA and other external factors of cells are evaluated at this checkpoint. If the conditions are inadequate, the cell will not be allowed to continue to the S phase.

•G_1 checkpoint is also known as the restriction point at which the cell irreversibly commits to the cell division process. Cell set up certain requirements to be fulfilled by the cell to pass the check points.

•External factor such as growth factors play a vital role in carrying the cell past the G_1 checkpoint. The cell will only pass the checkpoint if it has an appropriate size and has adequate energy reserves.

•At this point, the cell also checks for DNA damage.

•A cell that does not meet all the requirements will not progress to the S phase. Those cells halt the cycle and attempt to correct the problematic condition, or the cell may undergoes inactivation into G_0 phase and await for further signals when conditions improve.

•If a cell meets all the requirements for the G_1 checkpoint, the cell will enter S phase and begin DNA replication.

•This G1 checkpoint involves signaled by cyclins and cyclin dependent kinases (CDKs).

G_1 checkpoint (G_1 restriction point)

- This checkpoint is present at the end of the G_1 phase and before S phase.
- This checkpoint helps in taking the decision of whether the cell should divide, delay division, or enter a resting stage (Go phase). If there are unfavourable conditions for the cell division, then this restriction point restrict the progression to the next phase by passing the cell to Go phase for an extended period of time.
- This restriction point is mainly controlled by the action of the CKI-p16 (CDK inhibitor p16).The inhibited CDK not bind with cyclin D1, hence there is no cell progression.
- Active cyclin D-cdk complexes phosphorylate retinoblastoma protein (pRb) in the nucleus.
- Un-phosphorylated pRb acts as an inhibitor of G_1 by preventing E2F-mediated transcription.
- Once pRb gets phosphorylated, E2F activates the transcription of cyclins E and A, which then interacts with CDK2 to allow for G_1-S phase transition.
- This brings the cell to the end of the first checkpoint (unphosphorylated Rb inhibits the E2F).

G2 check point:

The G2 checkpoint ensures all of the chromosomes have been accurately replicated and that the replicated chromosome is not damaged before cell enters mitosis.

- G_2 checkpoint prevents the cell from entering into the mitotic phase if certain conditions are not met.

- If the checkpoint mechanisms detect problems with the DNA, the cell cycle is halted and the cell attempts to either complete DNA replication or repair the damaged DNA.

- If the DNA has been correctly replicated, cyclin dependent kinases (CDKs) signal the beginning of mitotic cell division

G_2 checkpoint

- This restriction point is located at the end of the G_2 phase.This checks the number of factors which are essential for the cell division.
- Maturation-promoting factor or mitosis promoting factor or M-phase promoting factor- (MPF) is a protein composed of cyclin-B and CDK-1. This protein promotes the G_2 phase into the entrance of M-phase. MPF is activated at the end of G_2 by a phosphatase (Chk) which removes an inhibitory phosphate group added earlier.

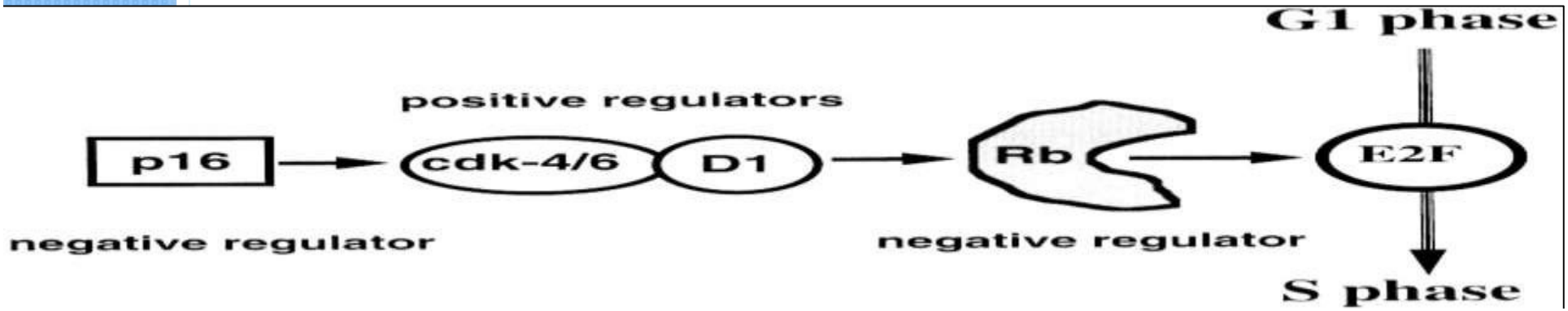
•The main functions of MPF in this restriction point are

a. Triggers the formation of mitotic spindle.

b. Promotes chromosome condensation.

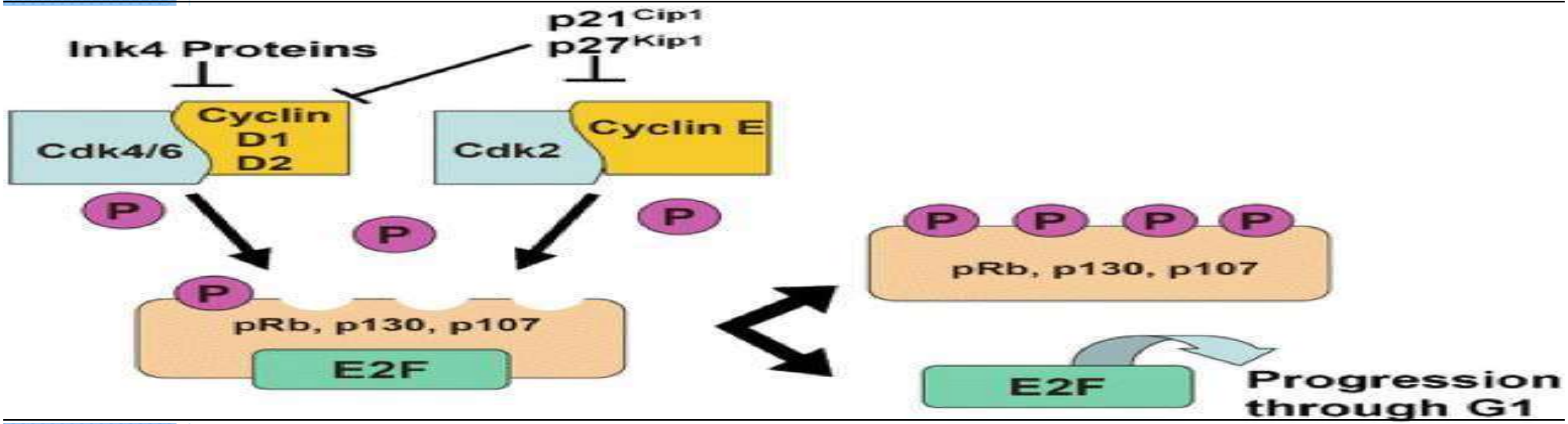
c. Causes nuclear envelop breakdown.

- If there are any damages are noticed in this restriction point, then the phasphatase not activate the MPF, resulting in the arrest of cell cycle in G_2 phase till the repair of the damaged DNA. This prevents the transfer of defected DNA into the daughter cells.



METAPHASE CHECKPOINT

- This occurs at metaphase.
- Anaphase-promoting complex (APC) regulates this checkpoint. This is also called spindle checkpoint.
- This checks whether all chromosomes are properly attached to the spindle or not. This also governs the alignment of the chromosomes and integrity of the spindles. If there are mistakes then it delays the cell in entering into anaphase from metaphase.



M check point:

•The M checkpoint occurs at the end of the metaphase of mitosis.

•M checkpoint determines whether all the sister chromatids are correctly attached to the spindle fiber before the cell enters the irreversible anaphase stage.

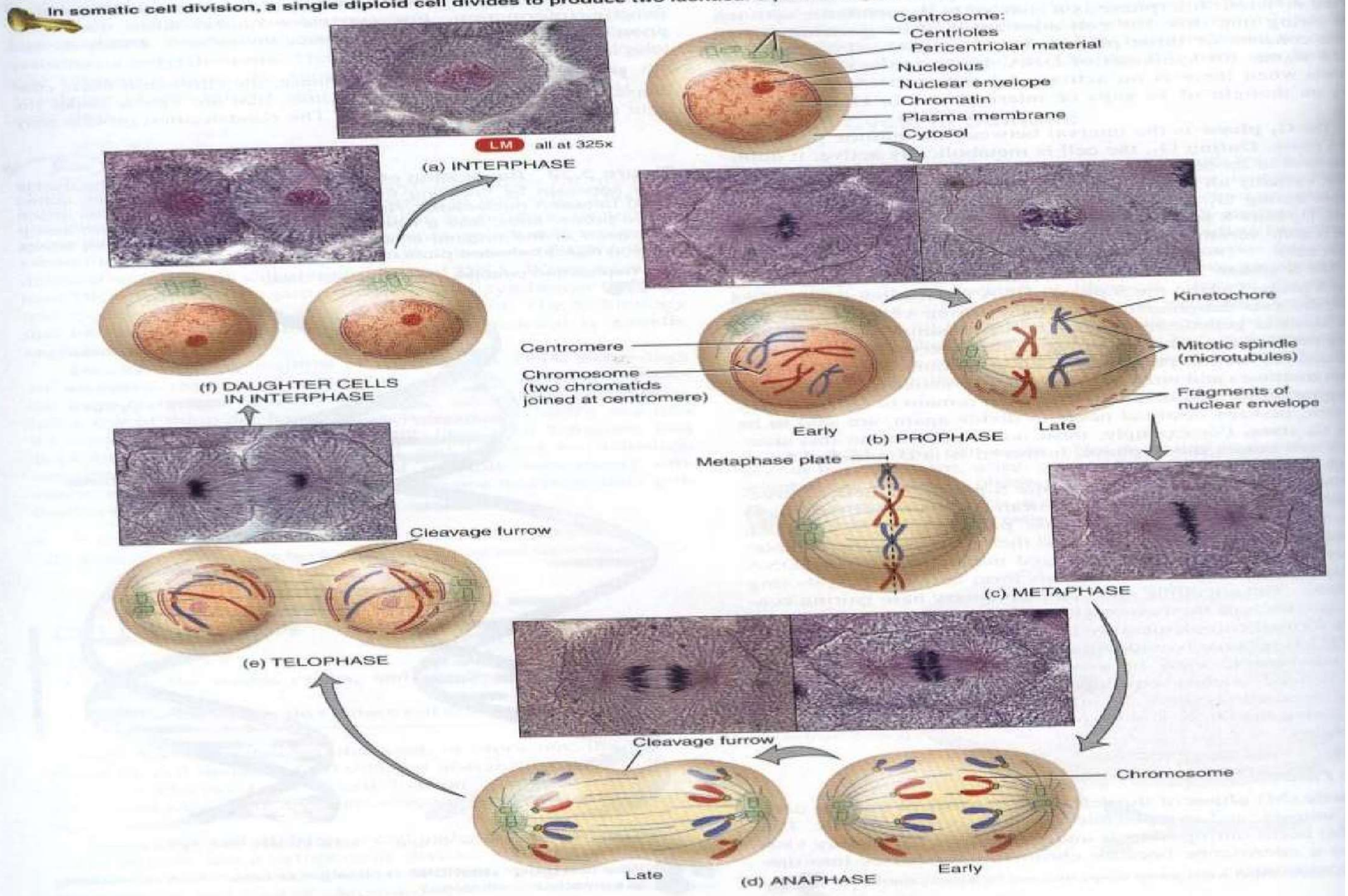
•M checkpoint is also known as the spindle checkpoint because it determines whether all the sister chromatids are correctly attached to the spindle microtubules or not.

•At the end stage of metaphase, spindle fiber arising from opposite pole of cell attached to kinetochore of centromere of sister chromatid in equatorial plane. Then the cell enter into anaphase which is characterized by separation of sister chromosome toward opposite pole. Since anaphase is irreversible step in cell cycle, M phase check point is very crucial which ensure proper attachment of spindle to sister chromatids.

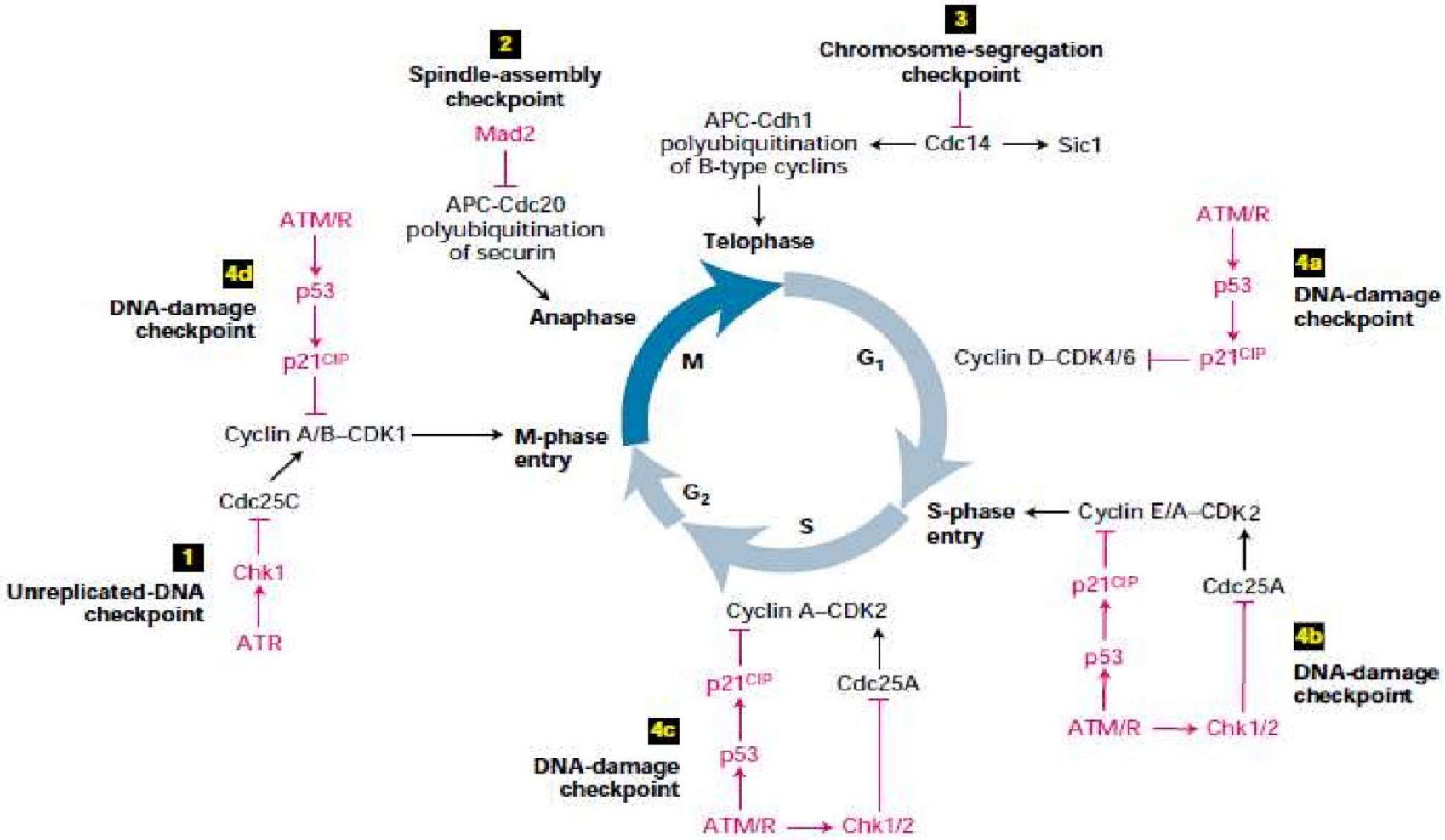
•M check point also involves signal from cyclin dependent kinases

Figure 3.33 Cell division: mitosis and cytokinesis. Begin the sequence at (a) at the top of the figure and read clockwise until you complete the process.

In somatic cell division, a single diploid cell divides to produce two identical diploid daughter cells.



When does cytokinesis begin?



▲ FIGURE 21-32 Overview of checkpoint controls in the cell cycle. The unreplicated-DNA checkpoint (**1**) prevents activation of cyclin A-CDK1 and cyclin B-CDK1 (i.e., mitosis-promoting factor, MPF) by activation of an ATR-Chk1 protein kinase cascade that phosphorylates and inactivates Cdc25C, thereby inhibiting entry into mitosis. In the spindle-assembly checkpoint (**2**), Mad2 and other proteins inhibit activation of the APC specificity factor (Cdc20) required for polyubiquitination of securin, thereby preventing entry into anaphase. The chromosome-segregation checkpoint (**3**) prevents release of the Cdc14 phosphatase from nucleoli, thereby blocking activation of the APC specificity factor (Cdh1) required for polyubiquitination of B-type cyclins as well as induction of Sic1. As a result, the decrease in MPF activity required for the events of telophase does not occur. In the initial phase of the DNA-damage checkpoint (**4**), the ATM or ATR protein kinase (ATM/R) is activated. The active kinases then trigger two pathways: the Chk-Cdc25A pathway (**4b** and **4c**), blocking entry into or through the S phase, and the p53-$p21^{CIP}$ pathway, leading to arrest in G_1, S, and G_2 (**4a**–**4b**). See text for further discussion. Red symbols indicate pathways that inhibit progression through the cell cycle.

KEY CONCEPTS OF SECTION : Checkpoints in Cell-Cycle Regulation

- Checkpoint controls function to ensure that chromosomes are intact and that critical stages of the cell cycle are completed before the following stage is initiated.
- The unreplicated-DNA checkpoint operates during S and G_2 to prevent the activation of MPF before DNA synthesis is complete by inhibiting the activation of CDK1 by Cdc25C.
- The spindle-assembly checkpoint, which prevents premature initiation of anaphase, utilizes Mad2 and other proteins to regulate the APC specificity factor Cdc20 that targets securinfor polyubiquitination .
- The chromosome-segregation checkpoint prevents telophase and cytokinesis until daughter chromosomes have been properly segregated, so that the daughter cell has a full set of chromosomes.
- In the chromosome-segregation checkpoint, the small GTPase Tem1 controls the availability of Cdc14 phosphatase, which in turn activates the APC specificity factor Cdh1 that targets B-type cyclins for degradation, causing inactivation of MPF.
- The DNA-damage checkpoint arrests the cell cycle in response to DNA damage until the damage is repaired. Three types of tumor-suppressor proteins (ATM/ATR, Chk1/2, and p53) are critical to this checkpoint.
- Activation of the ATM or ATR protein kinases in response to DNA damage due to UV light or –irradiation leads to arrest in G1 and the S phase via a pathway that leads to loss of Cdc25A phosphatase activity. A second pathway from activated ATM/R stabilizes p53, which stimulates expression of $p21^{CIP}$. Subsequent inhibition of multiple CDK-cyclin complexes by $p21^{CIP}$ causes prolonged arrest in G_1 and G_2.
- In response to extensive DNA damage, p53 also activates genes that induce apoptosis.

Regulation of cell cycle:

The cell cycle is controlled by regulator molecules that either promote the process or stop it from progressing

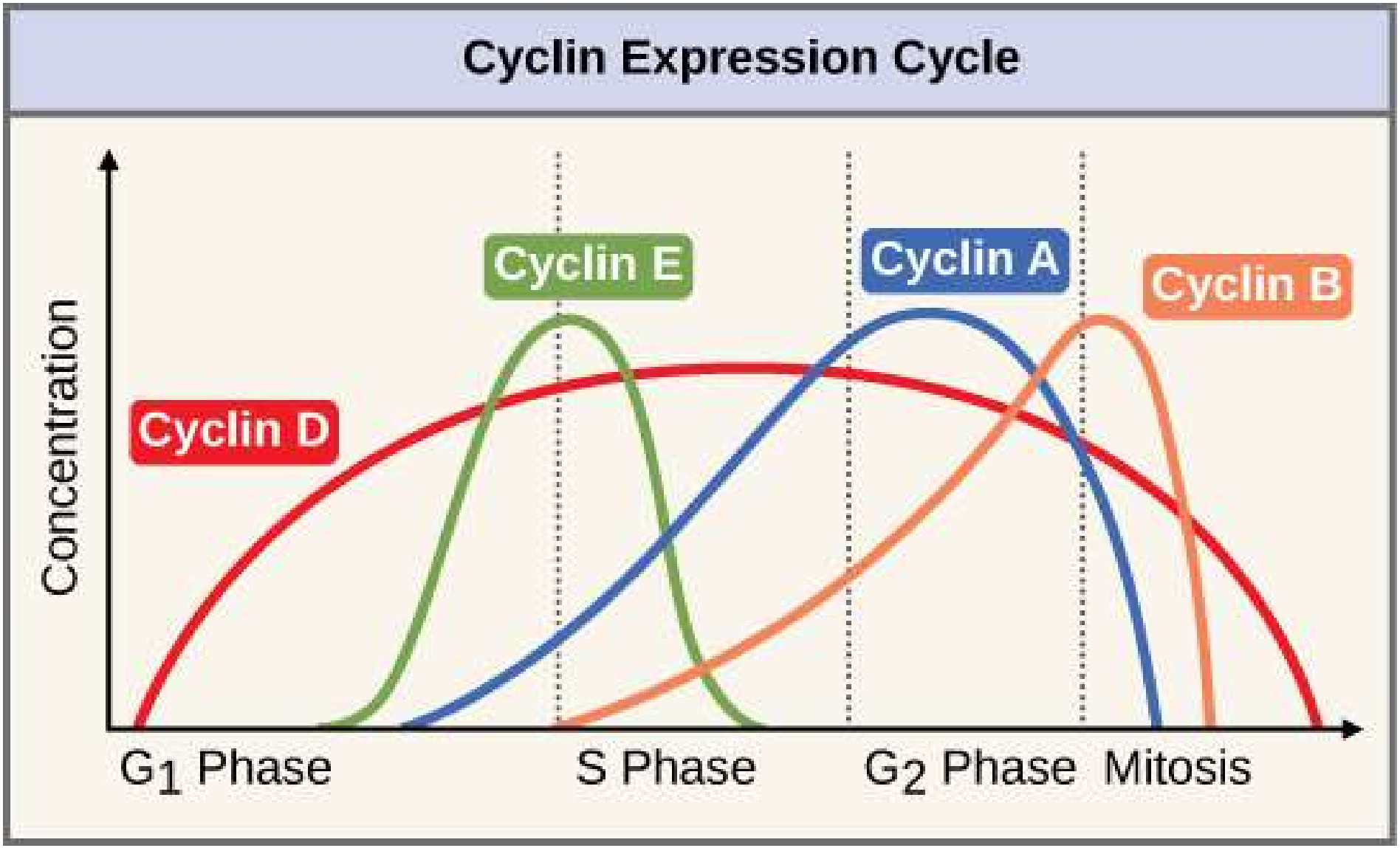
CELL CYCLE REGULATORS

The cell cycle is regulated by

1. **cyclins**
2. **Cyclin-dependent kinases (CDKs)**
3. **cyclin-dependent kinase inhibitors (CDKIs).**

1. CYCLINS:

- Their concentration varies during the cell cycle. Cyclins are the family of proteins which regulates the cell cycle.
- There are several types of cyclins that are active in different parts of the cell cycle and causes phosphorylation of CDK.
- There are also several "orphan" cyclins for which no CDK partner has been identified.
- For example, cyclin F is an orphan cyclin that is essential for G2/M transition.
- There are two main groups of cyclins.


Cyclin Expression Cycle
Concentration
Cyclin D
Cyclin E
Cyclin A
Cyclin B
G1 Phase
S Phase
G2 Phase
Mitosis

A. G1/S CYCLINS: Examples- Cyclin A,D and E.

- These cyclins are essential for the control of the cell cycle at the G_1/S transition.
- Cyclin A / CDK 2- active in S phase. Cyclin A binds to S phase CDK 2 and is required to progress through the S Phase. Cyclin A/CDK 2 is inhibited by the complex $p^{21\ CIP}$.
- The un-phophorylated form of Rb binds with E2F family of transcription factors which controls expression of several genes involved in cell cycle progression (example-cyclin-E).
- Rb acts as a repression, so in complex with E2F it prevents the expression of E2F genes, and this inhibits the cell progression from G_1 to S phase.
- The binding of cyclin D/CDK4 and cyclin D/CDK 6 lead to partial phosphorylation of Rb, by reducing its binding to E2F. The E2F gene activates the expression of cyclin E bind with CDK 2 and causes complete phosphorylation of Rb. This progresses the cell cycle from G_1 to S phase.

B.A.G2/M CYCLINS: Example; cyclin B/ CDK1.

- These are essential for the control of the cell cycle at the G_2/M transition.
- G2/M cyclins accumulate steadily during G_2 and are abruptly destroyed as cells exit from mitosis (at the end of the M-Phase).
- Cyclin B/ CDK1- regulates progression from G_2 to M Phase. Cyclin B is a mitotic cyclin. The amount of cyclin B (which binds to CDK1) and the activity of cyclin B-CDK complex rise through the cell cycle until mitosis. The cyclinB-CDK1 complex is called maturation promoting factor (MPF).
- There are two types of cyclin B.

CYCLIN B

1. CYCLIN B1/CDK1

- This complex is involved in the early events of mitosis such as chromosome condensation, nuclear envelop breakdown.
- This complex is localized in the microtubules.

2. CYCLIN B2/CDK1

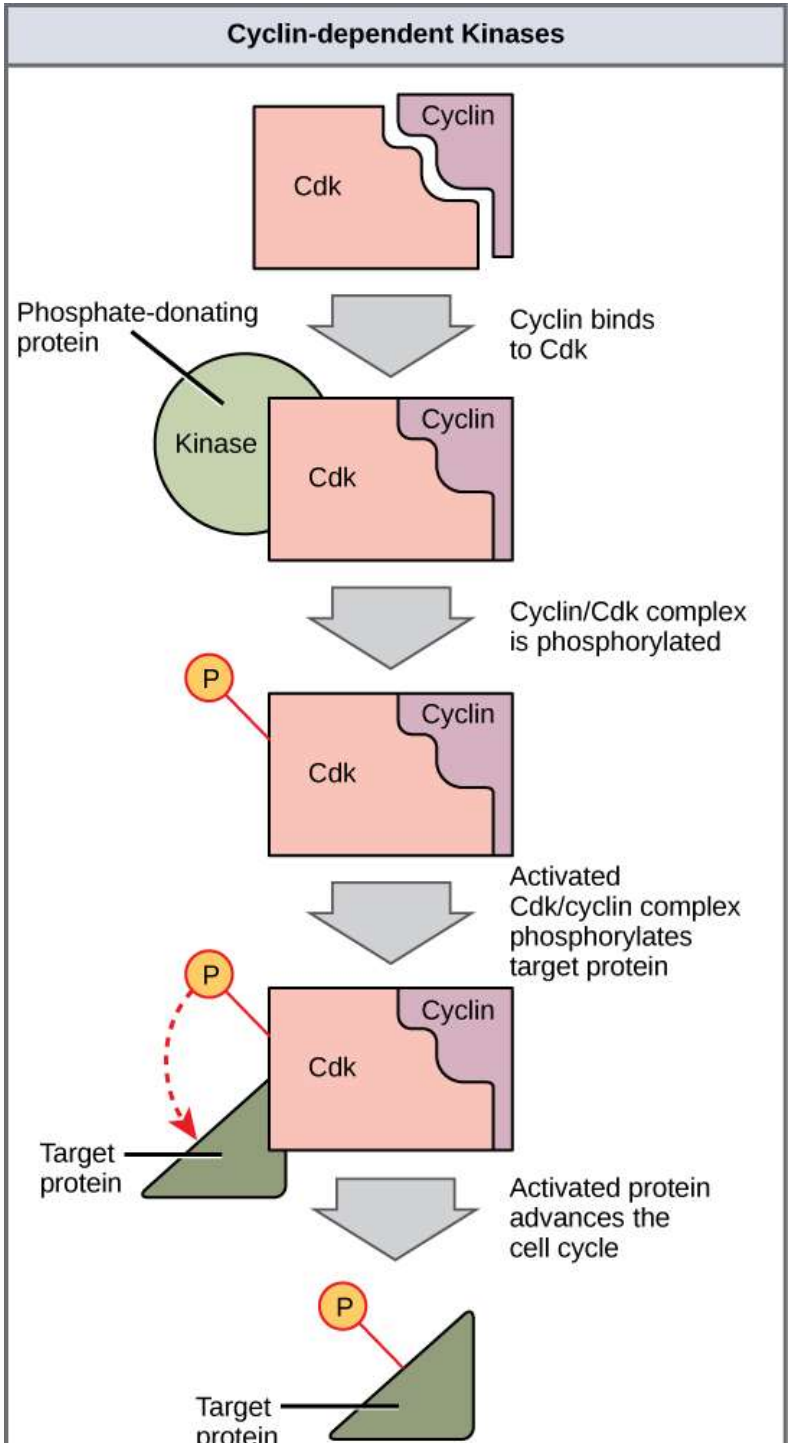
- This complex is localized in the Golgi apparatus.
- Cyclin B2 is also binds to a gene-transforming growth factor (TGF beta receptor 2). This gene complex transcribes several genes required for the cell proliferation.

CYCLIN-DEPENDENT KINSASES (CDKS)

- These are a family of protein kinases that regulates the cell cycle.
- They are present in all known eukaryotic cells.

PHASE	CYCLIN	CDK
Go	C	CDK3
GI	D,E	CDK4, CDK2,CDK6
S	A,E	CDK2
G2	A	CDK2, CDK1
M	B	CDK 1

Cyclin-dependent kinases (Cdks) are protein kinases that, when fully activated, can phosphorylate and thus activate other proteins that advance the cell cycle past a checkpoint. To become fully activated, a Cdk must bind to a cyclin protein and then be phosphorylated by another kinase



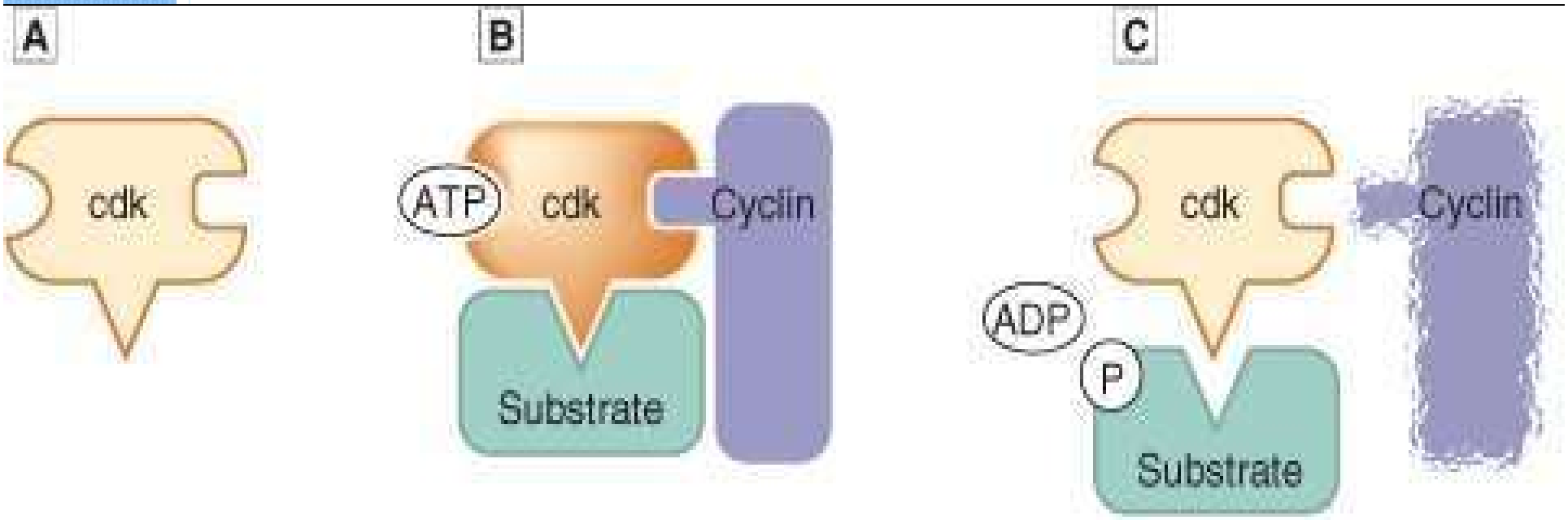
CYCLIN-DEPENDENT KINASE INHIBITORS (CDKIS)

- CDKI is a protein which inhibits cyclin-dependent kinase (CDK).
- Cell cycle progression is negatively controlled by cyclin-dependent kinases inhibitors (called CDIs, CKIs or CDKIs).
- These are involved in cell cycle arrest at the G_1 phase.

CDK I	INTERACTING CDK
p 16	CDK 4,CDK6
p 15	CDK4
p 18	CDK4,CDK6
p 19	CDK4,CDK6
p 21	CYCLIN E1, CDK2
p 27	CDK3,CDK4,CDK2,CYCLIN E1
p 57	CYCLIN E1, CDK2

POSITIVE REGULATORS

- Are those which control the changes necessary for cell division.
- They include:-
  - **Cyclins**
  - **Cyclin-dependent kinases(cdks)**
  - **Polo-like kinases**



NEGATIVE REGULATORS

- Are those which control the positive regulators.

•Negative regulators halt the cell cycle.

•Negative regulatory molecules are retinoblastoma protein (Rb), p53, and p21.

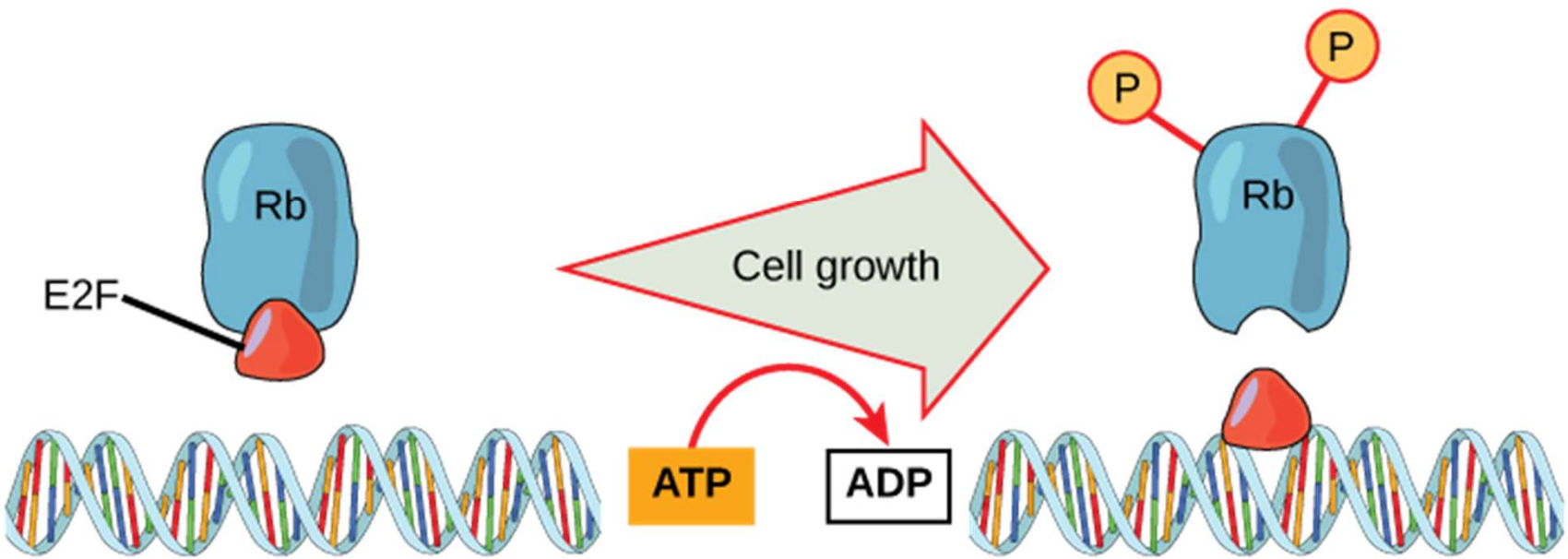
•If negative regulator proteins are damaged or become non-functional then it results in uncontrolled cell division leading to tumor or cancer.

- They include :-
  - **Rb proteins**
  - **P_{53} gene**
  - **Inhibitors of cdks** - which are of 2 types

- **Ink family (Inhibitors of kinases) = P_{19} ,P_{15}**

- **CIP family (cdks inhibitory proteins)= P_{21}, P_{57}**

Rb Regulation of the Cell



Unphosphorylated Rb binds transcription factor E2F. E2F cannot bind the DNA, and transcription is blocked.

Cell growth triggers the phosphoryation of Rb. Phosphorylated Rb releases E2F, which binds the DNA and turns on gene expression, thus advancing the cell cycle.

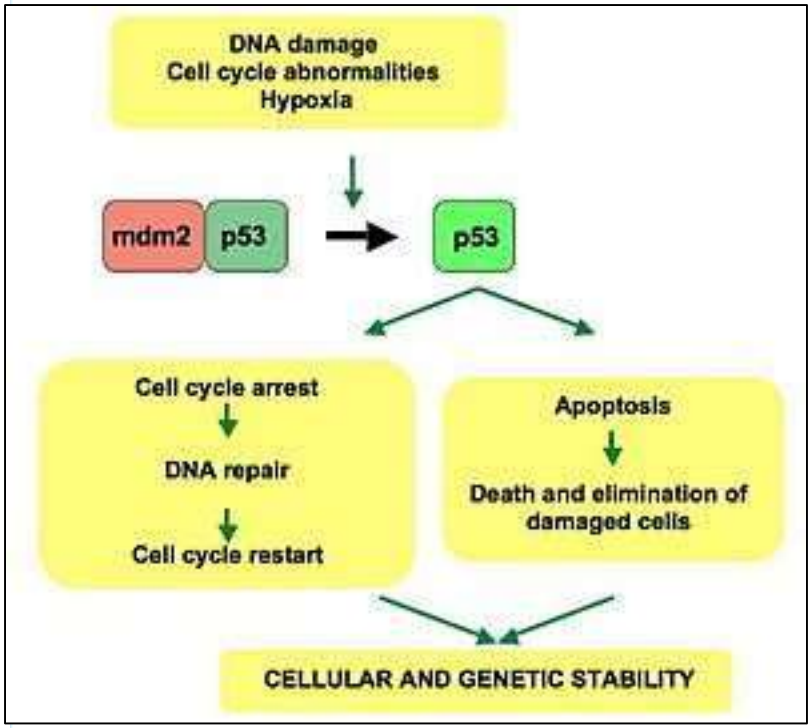
Rb PROTEIN

- **Rb protein was initially identified as the product of the prototype tumor-suppressor gene, *RB*.**
- The products of tumor-suppressor genes function in various ways to inhibit progression through the cell cycle (loss-of-function mutations in *RB* are associated with the disease *hereditary retinoblastoma.)*
- A child with this disease inherits one normal RB^+ allele from one parentand one mutant RB^- allele from the other. If the RB^+ allele in any of the trillions of cells that make up the human body becomes mutated to a RB^- allele, then no functional protein is expressed and the cell or one of its descendants is likely to become cancerous. Also, in most human cancer cells Rb function is inactivated, either by mutations in both alleles of *RB*, or by abnormal regulation of Rb phosphorylation.
- Rb protein is one of the most significant substrates of mammalian G1 cyclin-CDK complexes. Phosphorylation of Rb protein at multiple sites prevents its association with E2Fs, thereby permitting E2Fs to activate transcription of genes required for entry into S phase.

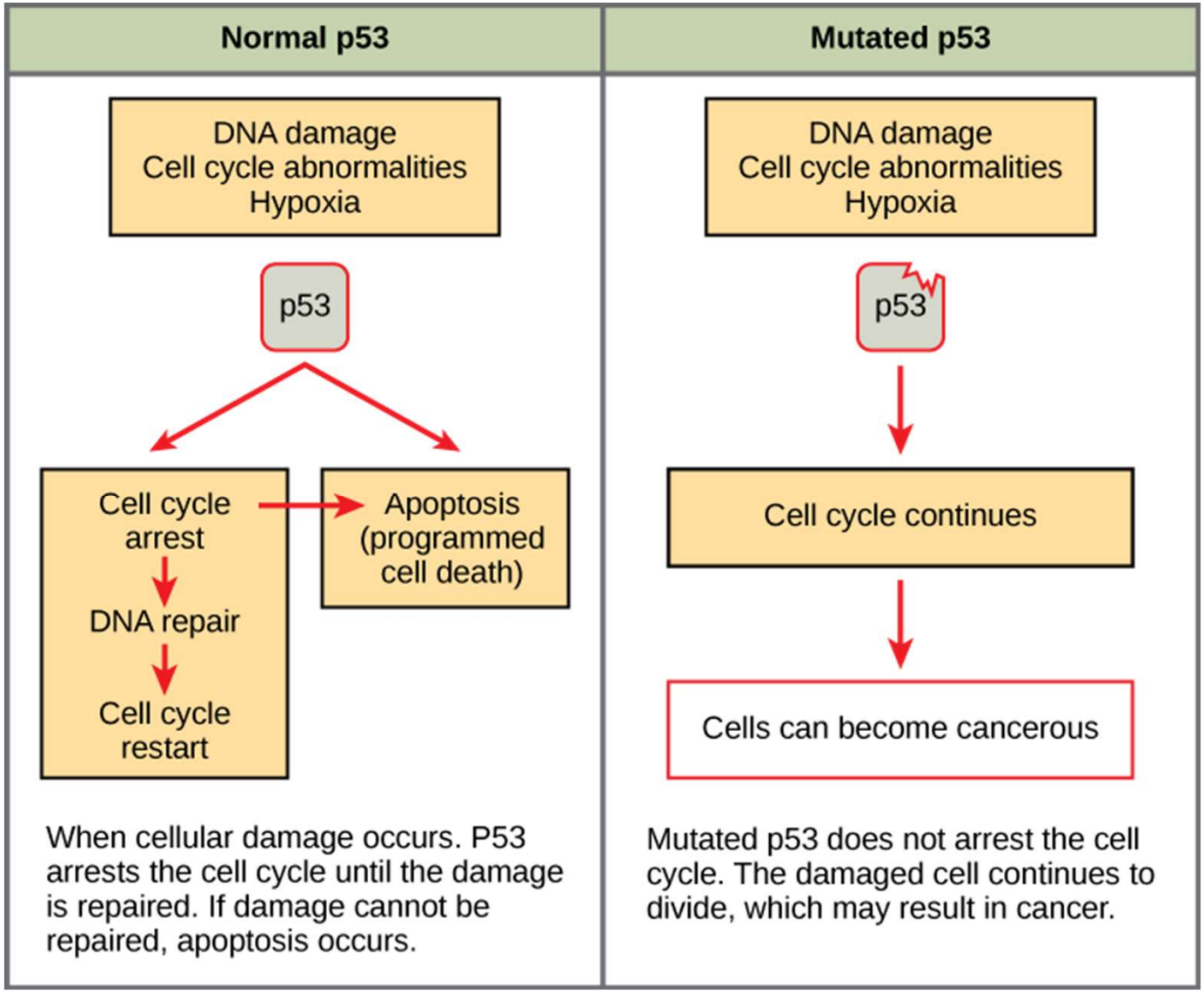
- **E2F stimulates its own expression and that of cyclin E and CDK2, positive cross-regulation of E2F and cyclin E-CDK2 produces a rapid rise of both activities in late G_1.**
- They accumulate, S-phase cyclin-CDK and mitotic cyclin-CDK complexes maintain Rb protein in the phosphorylated state throughout the S, G_2,and early M phases.
- After cells complete anaphase and enter early G_1 or G_0, the fall in cyclin-CDK levels leads to dephosphorylation of Rb by unopposed phosphatases. As a consequence, hypophosphorylated Rb is available to inhibit E2F activity during early G1 of the next cycle and in G0-arrested cells.
- **Unphosphorylated Rb protein binds to E2Fs, converting them into transcriptional repressors.**
- **Phosphorylation of Rb by cyclin D-CDK4/6 in mid G1 liberates E2Fs to activate transcription of genes encoding cyclin E, CDK2, and other proteins required for the S phase.**

p53 PROTEIN

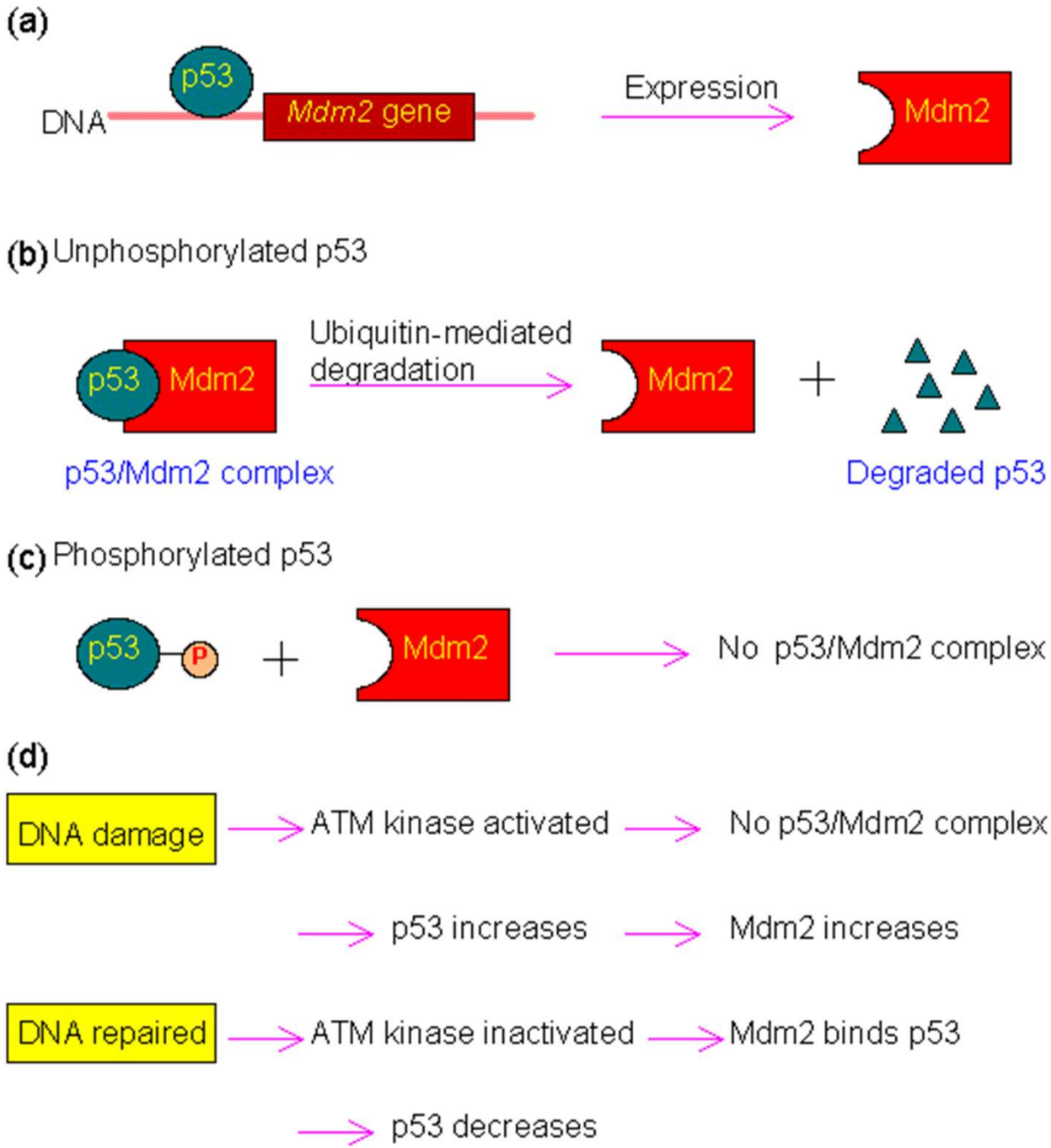
- DNA damage leads to the activation of the gene regulatory protein p53, which stimulates the transcription of several genes.
- One of these encodes a CKI proteins p21,which binds to G_1/S-Cdk and S-Cdk and inhibits their activites, thereby helping to block entry into S phase.



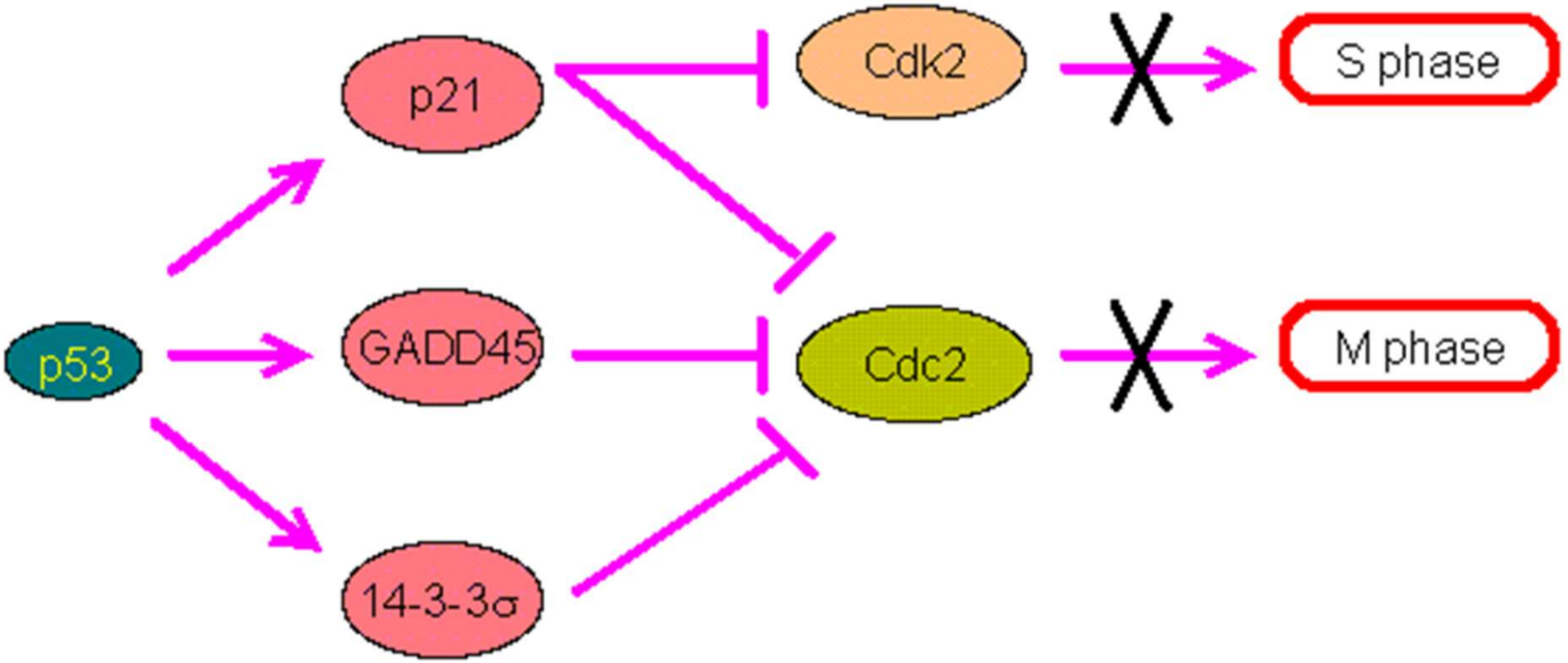
In a normal cell p53 is inactivated by its negative regulator, mdm2. Upon DNA damage or other stresses, various pathways will lead to the dissociation of the p53 and mdm2 complex. Once activated, p53 will induce a cell cycle arrest to allow either repair and survival of the cell or apoptosis to discard the damaged cell. How p53 makes this choice is currently unknown.



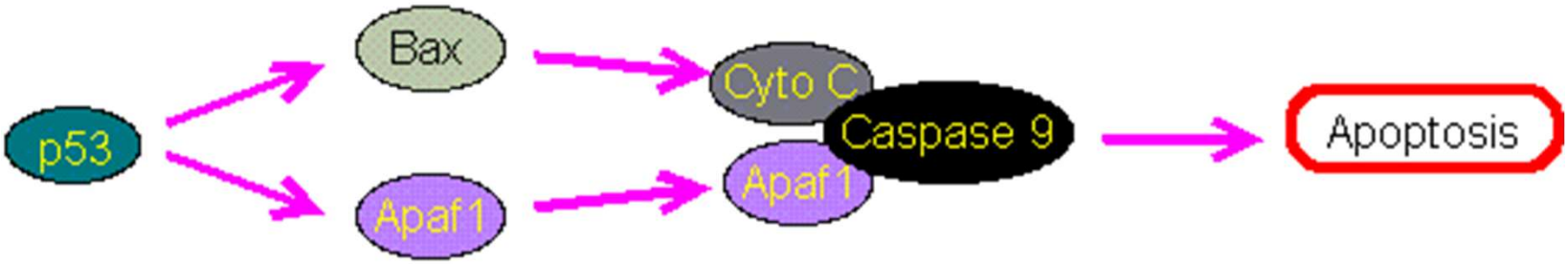
The role of normal p53 is to monitor DNA and the supply of oxygen (hypoxia is a condition of reduced oxygen supply). If damage is detected, p53 triggers repair mechanisms. If repairs are unsuccessful, p53 signals apoptosis. A cell with an abnormal p53 protein cannot repair damaged DNA and thus cannot signal apoptosis. Cells with abnormal p53 can become cancerous.


(a)
p53
DNA
Mdm2 gene
Expression
Mdm2
(b) Unphosphorylated p53
p53
Mdm2
Ubiquitin-mediated degradation
Mdm2
p53/Mdm2 complex
Degraded p53
(c) Phosphorylated p53
p53
P
Mdm2
No p53/Mdm2 complex
(d)
DNA damage
ATM kinase activated
No p53/Mdm2 complex
p53 increases
Mdm2 increases
DNA repaired
ATM kinase inactivated
Mdm2 binds p53
p53 decreases

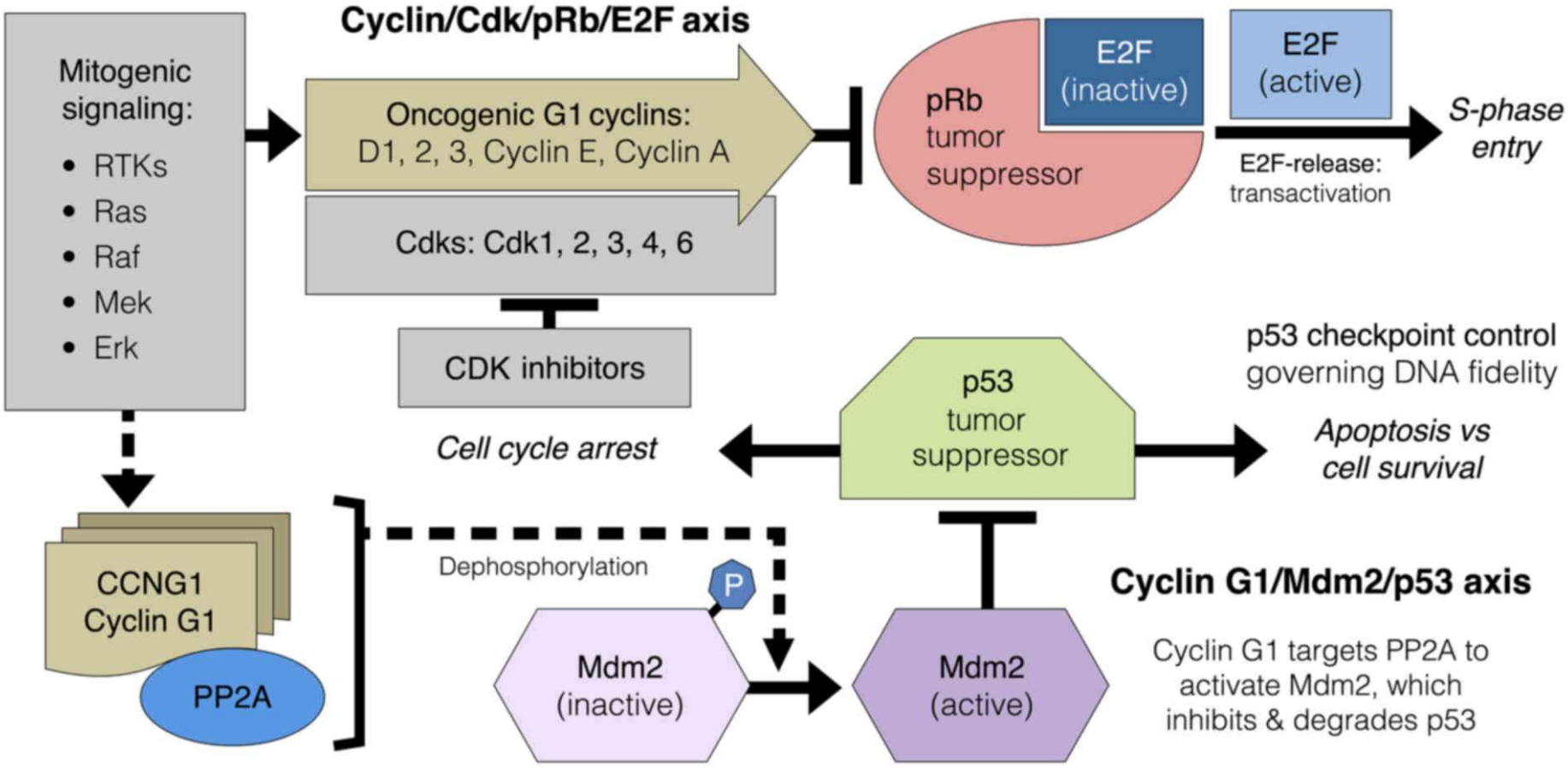
(a) Growth Arrest



(b) Apoptosis



CYCLINS: Abrogate key tumor suppressors governing cell cycle progression



INHIBITORS OF CDKS

- The activities of mammalian cyclin-CDK complexes also are regulated by CDK inhibitors (CIPs), which bind to and inhibit each of the mammalian **cyclin-CDK complexes, and INK4 proteins,** which block passage through G1 by specifically inhibiting CDK4 and CDK6.

CIP FAMILY (CDKS INHIBITORY PROTEINS)

- Three related CIPs—**$p21^{CIP}$, $p27^{KIP2}$, and $p57^{KIP2}$**—inhibit cyclin A-CDK2 activity and must be degraded before DNA replication can begin.
- $p21^{CIP}$ plays a role in the response of mammalian cells to DNA damage.

INK FAMILY (INHIBITORS OF KINASES)

- A second class of cyclin-CDK inhibitors called *INK4s* (*in*hibitors of *k*inase *4*) includes several small, closely related proteins that interact only with CDK4 and CDK6 and thus function specifically in controlling the mid-G1 phase.
- Binding of INK4s to CDK4/6 blocks their interaction with cyclin D and hence their protein kinase activity. The resulting decreased phosphorylation of Rb protein prevents transcriptional activation by E2Fs and entry into the S phase. One INK4 called p16 is a tumor suppressor.

p21

•p21 enforces the halt in the cell cycle dictated by p53 by binding to and inhibiting the activity of the Cdk/cyclin complexes.

•In case of DNA damage condition or inadequate cell size, more and more p53 and p21 are produced which halt the cell cycle and prevent the cell to enter S phase.

•These negative regulators are known as tumor suppressor protein and gene that codes for such proteins are called tumor suppressor gene.

•Tumor suppressor either halt the cell until repair or leads to apoptosis thus preventing damaged cell from division. If mutation occurs in tumor suppressor gene, then those negative regulator proteins lost the function to halt the cell cycle leading cancerous cell of continuous growth and division.

Table 17–2 **Summary of the Major Cell-Cycle Regulatory Proteins**

GENERAL NAME	FUNCTIONS AND COMMENTS
Protein kinases and protein phosphatases that modify Cdks	
Cdk-activating kinase (CAK)	phosphorylates an activating site in Cdks
Wee1 kinase	phosphorylates inhibitory sites in Cdks; primarily involved in suppressing Cdk1 activity before mitosis
Cdc25 phosphatase	removes inhibitory phosphates from Cdks; three family members (Cdc25A, B, C) in mammals; primarily involved in controlling Cdk1 activation at the onset of mitosis
Cdk inhibitor proteins (CKIs)	
Sic1 (budding yeast)	suppresses Cdk1 activity in G_1; phosphorylation by Cdk1 at the end of G_1 triggers its destruction
p27 (mammals)	suppresses G_1/S-Cdk and S-Cdk activities in G_1; helps cells withdraw from cell cycle when they terminally differentiate; phosphorylation by Cdk2 triggers its ubiquitylation by SCF
p21 (mammals)	suppresses G_1/S-Cdk and S-Cdk activities following DNA damage
p16 (mammals)	suppresses G_1-Cdk activity in G_1; frequently inactivated in cancer
Ubiquitin ligases and their activators	
APC/C	catalyzes ubiquitylation of regulatory proteins involved primarily in exit from mitosis, including securin and S- and M-cyclins; regulated by association with activating subunits
Cdc20	APC/C-activating subunit in all cells; triggers initial activation of APC/C at metaphase-to-anaphase transition; stimulated by M-Cdk activity
Cdh1	APC/C-activating subunit that maintains APC/C activity after anaphase and throughout G_1; inhibited by Cdk activity
SCF	catalyzes ubiquitylation of regulatory proteins involved in G_1 control, including some CKIs (Sic1 in budding yeast, p27 in mammals); phosphorylation of target protein usually required for this activity