C4 T – CELL BIOLOGY
UNIT 7: Cell Division

Cell cycle & its regulation

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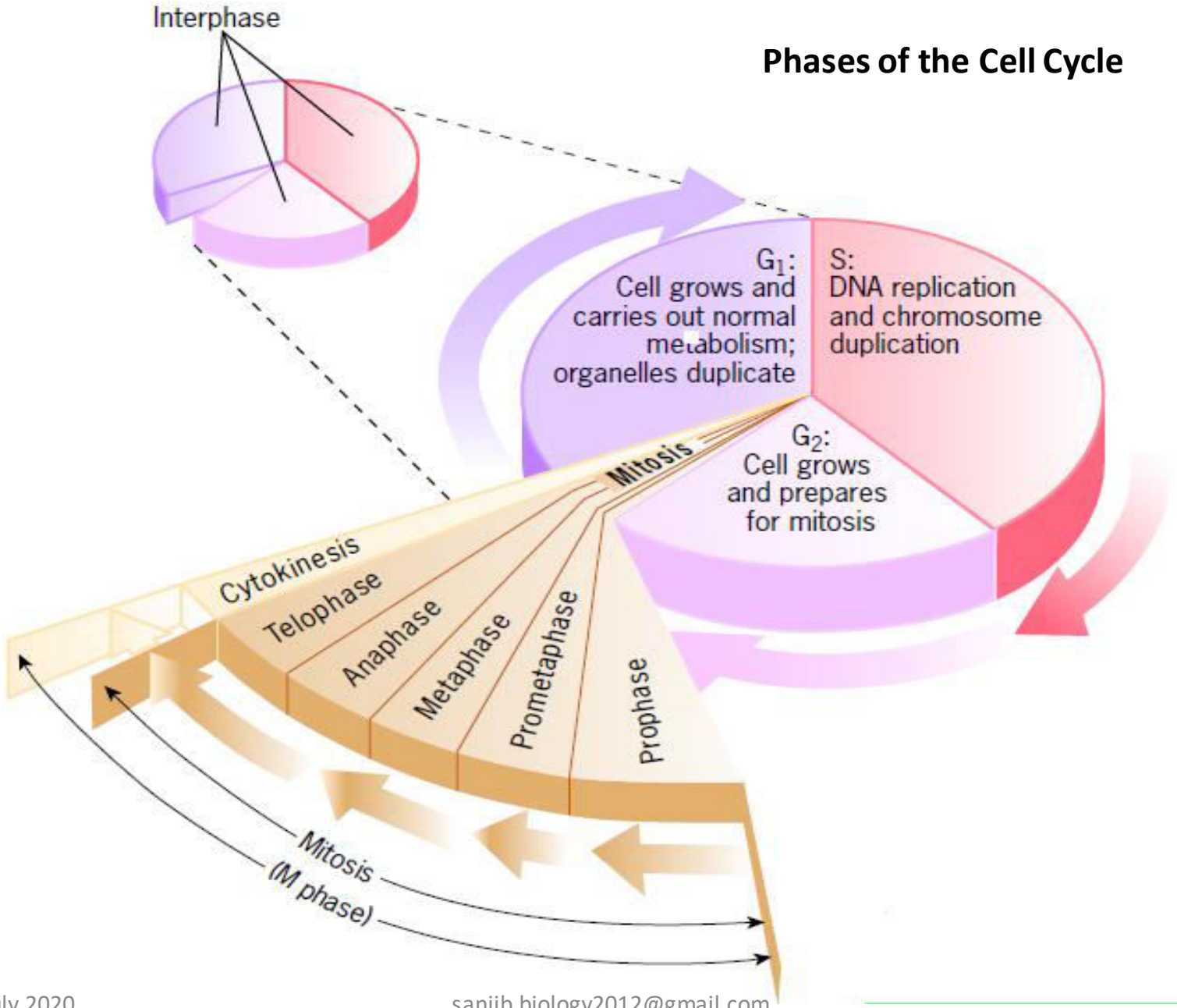
Phases of the Cell Cycle



Cell Cycles in Vivo

- ***Cells, that are highly specialized and lack the ability to divide***

  Eg: nerve cells, muscle cells, or red blood cells,
- ***Cells that normally do not divide but can be induced to begin DNA synthesis and divide when given an appropriate stimulus.***

  Eg: Liver cells and Lymphocytes
- ***Cells that normally possess a relatively high level of mitotic activity.***

  Eg: stem cells of adult tissue

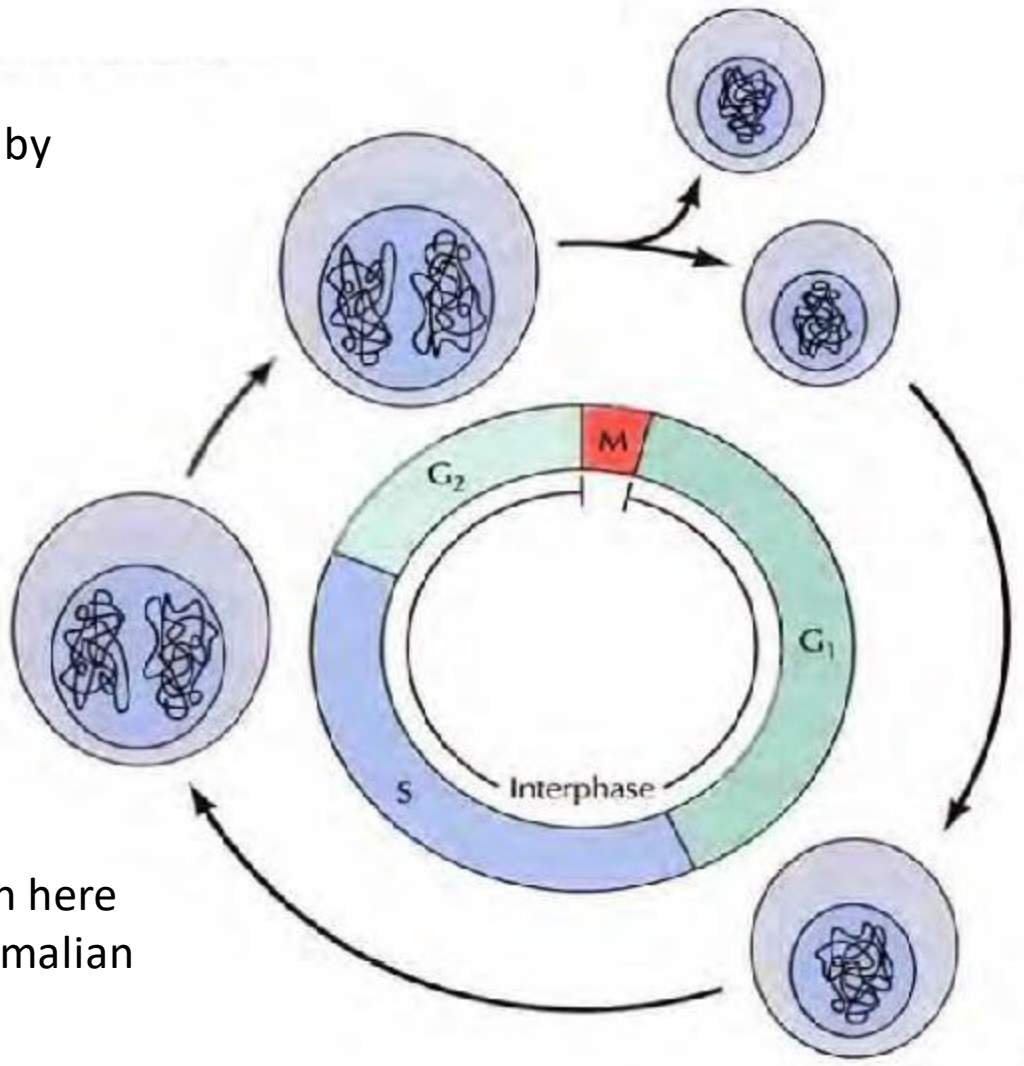
Phases of the cell cycle

The division cycle of most eukaryotic cells is divided into four discrete phases: M, G1, S, and G2.

M phase (mitosis) is usually followed by cytokinesis.

S phase is the period during which DNA replication occurs.

The cell grows throughout **interphase**, which includes *G1, S, and G2*

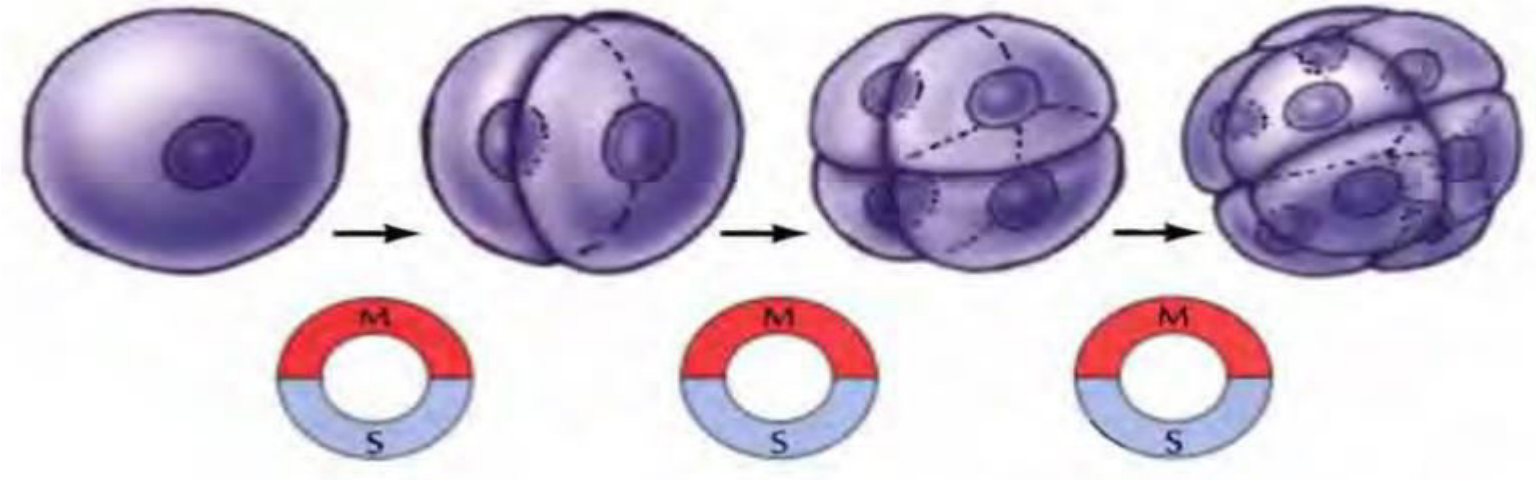


• *The relative* lengths of the cell cycle phases shown here are typical of rapidly replicating mammalian cells.

Duration of cell cycle

- The phases varies considerably in different kinds of cells.
- For a typical rapidly proliferating human cell with a total cycle time of 24 hours, the G1 phase might last about 11 hours, S phase about 8 hours, G2 about 4 hours, and M about 1 hour.
- Other types of cells, however, can divide much more rapidly.
- Budding yeasts, for example, can progress through all four stages of the cell cycle in only about 90 minutes.
- Even shorter cell cycles (30 minutes or less) occur in early embryo cells shortly after fertilization of the egg.

Embryonic cell cycles



Early embryonic cell cycles rapidly divide the cytoplasm of the egg into smaller cells. The cells do not grow during these cycles, which lack G1 and G2 and consist simply of short S phases alternating with M phase

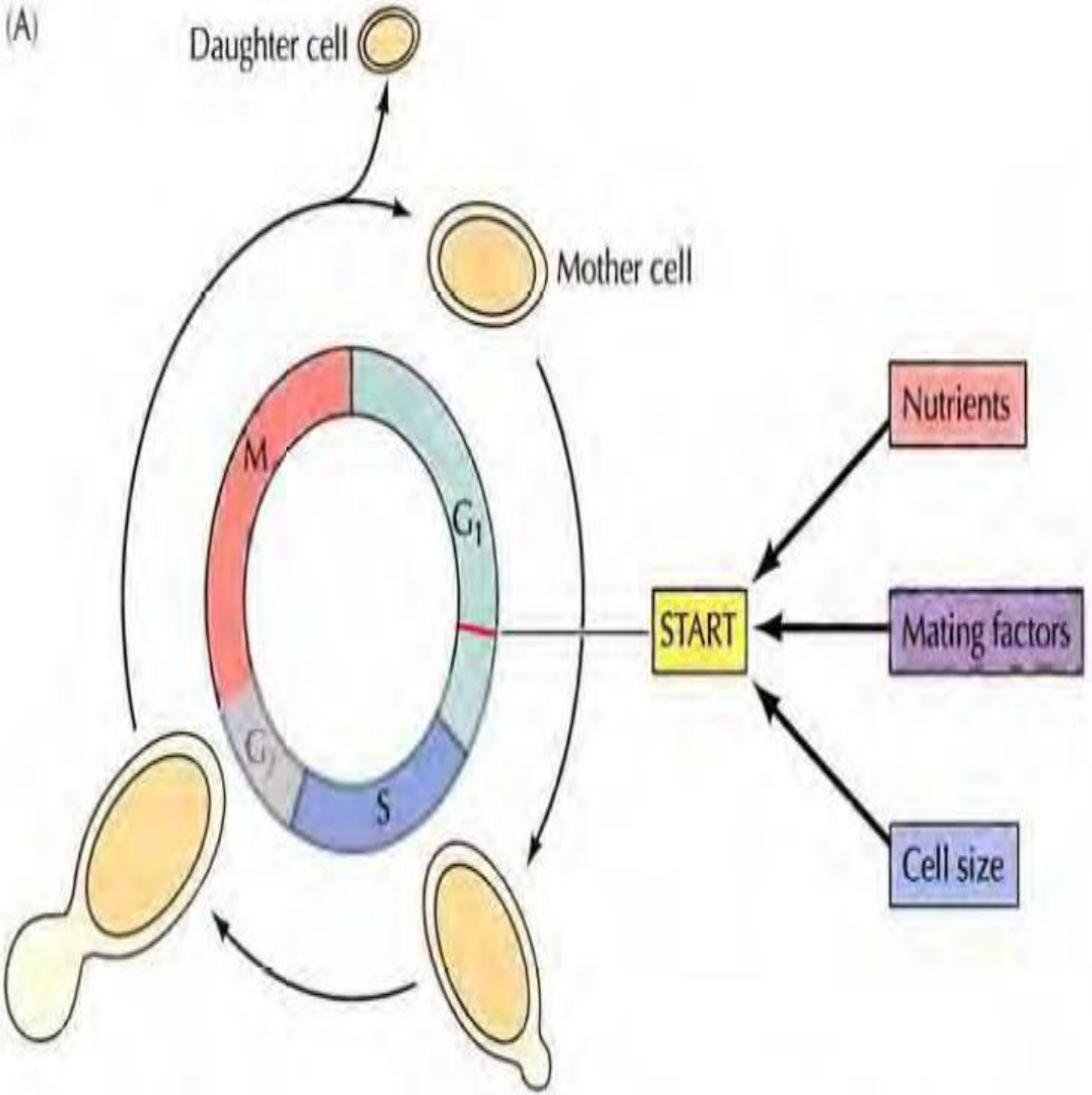
Regulation of the Cell Cycle by Cell Growth and Extracellular Signals

- progression of cells through the division cycle is regulated by
- Extracellular signals (effect of growth factors on animal cell) from the environment, as well as by internal signals
- that monitor and coordinate the various processes (cell growth, DNA replication, mitosis) that take place during different cell cycle phases.

START:

- In budding yeast *(Saccharomyces cerevisiae),* major cell cycle regulatory point occurs late in G1 and controls progression from G1 to S.
- Once cells have passed **START**, they are committed to entering **S phase** and undergoing one cell division cycle.
- passage through START is a highly regulated event in the yeast cell cycle where it is controlled by external signals, such as the availability of nutrients, as well as by cell size.

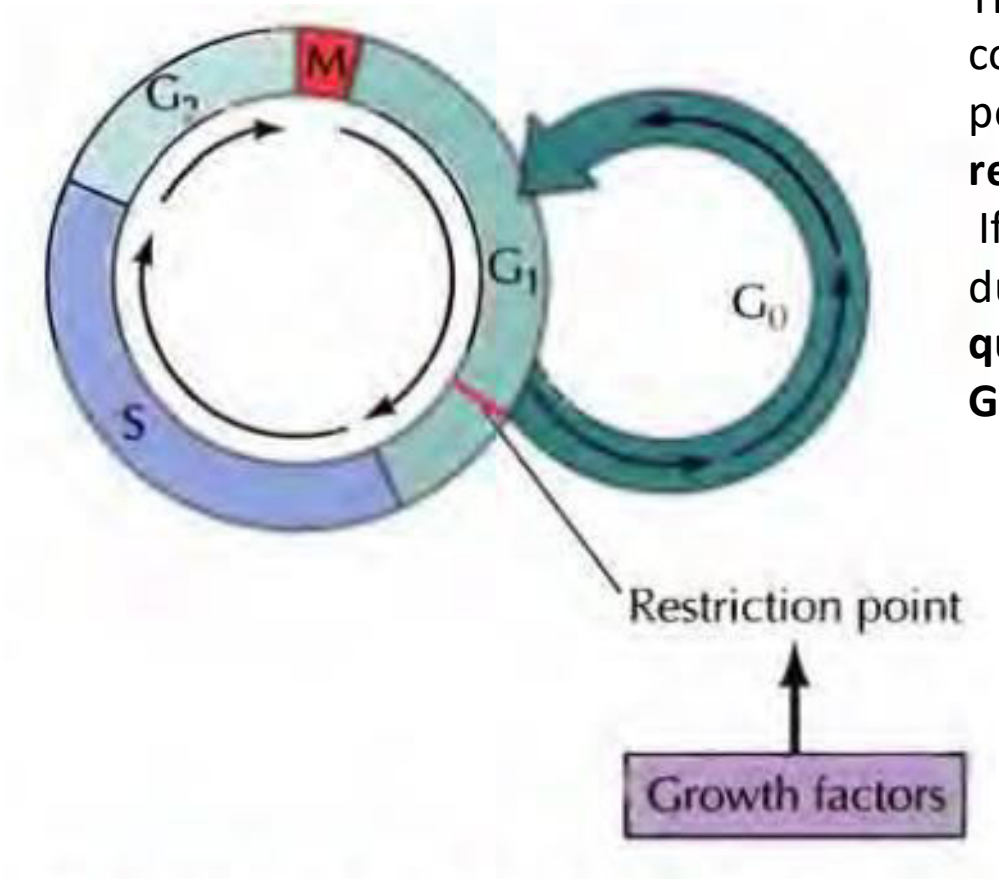
- For example
- if yeasts are faced with a shortage of nutrients, they arrest their cell cycle at START and enter a resting state rather than proceeding to S phase.
- Thus START represents a decision point at which the cell determines whether sufficient nutrients are available to support progression through the rest of the division cycle


(A)
Daughter cell
Mother cell
M
G1
G2
S
START
Nutrients
Mating factors
Cell size

RESTRICTION POINT

- proliferation of most animal cells is similarly regulated in the G1 phase of the cell cycle. a decision point in late G1, called the restriction point in animal cells analogously to **START** in yeasts
- passage of animal cells through the cell cycle is regulated primarily by the extracellular growth factors that signal cell proliferation, rather than by the availability of nutrients

Regulation of animal cell cycles by growth factors



The availability of growth factors control the animal cell cycle at a point in late G1 called the **restriction point**.

If growth factors are not available during G1, the cells enter a **quiescent stage** of the cycle called **G0.**

G0

- if appropriate growth factors are not available in *G1 , progression* through the cell cycle stops at the restriction point.
- Such arrested cells then enter a quiescent stage of the cell cycle called G0 in which they can remain for long periods of time without proliferating.
- G0 cells are metabolically active, although they cease growth and have reduced rates of protein synthesis.
- As already noted, many cells in animals remain in G0 unless called on to proliferate by appropriate growth factors or other extracellular signals.

- **For example**, **skin fibroblasts** are arrested in G0 until they are stimulated to divide as required to repair damage resulting from a wound. The proliferation of these cells is triggered by platelet-derived growth factor, which is released from blood platelets during clotting and signals the proliferation of fibroblasts in the vicinity of the injured tissue.

Cell Cycle Checkpoints

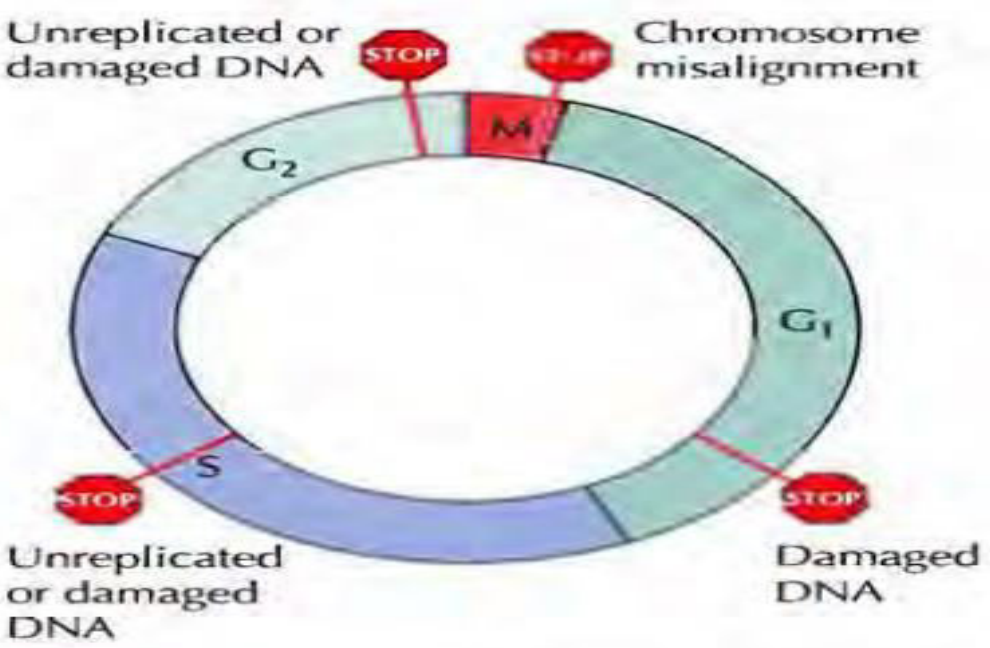


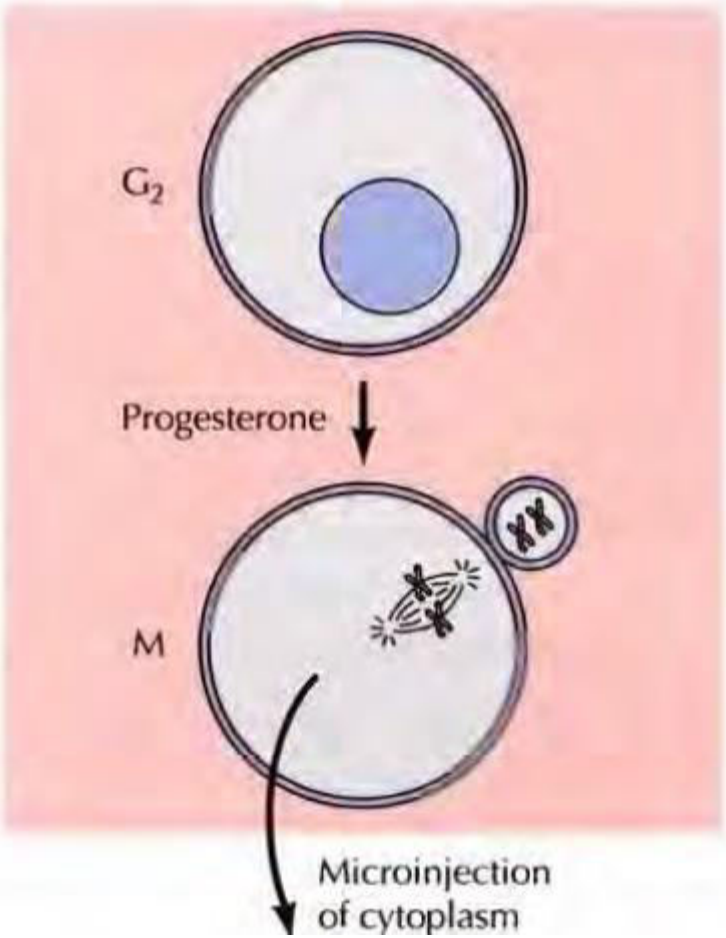
FIGURE 16.8 Cell cycle checkpoints Several checkpoints function to ensure that complete genomes are transmitted to daughter cells. DNA damage checkpoints in G_1, S, and G_2 lead to cell cycle arrest in response to damaged or unreplicated DNA. Another checkpoint, called the spindle assembly checkpoint, arrests mitosis if the chromosomes are not properly aligned on the mitotic spindle.

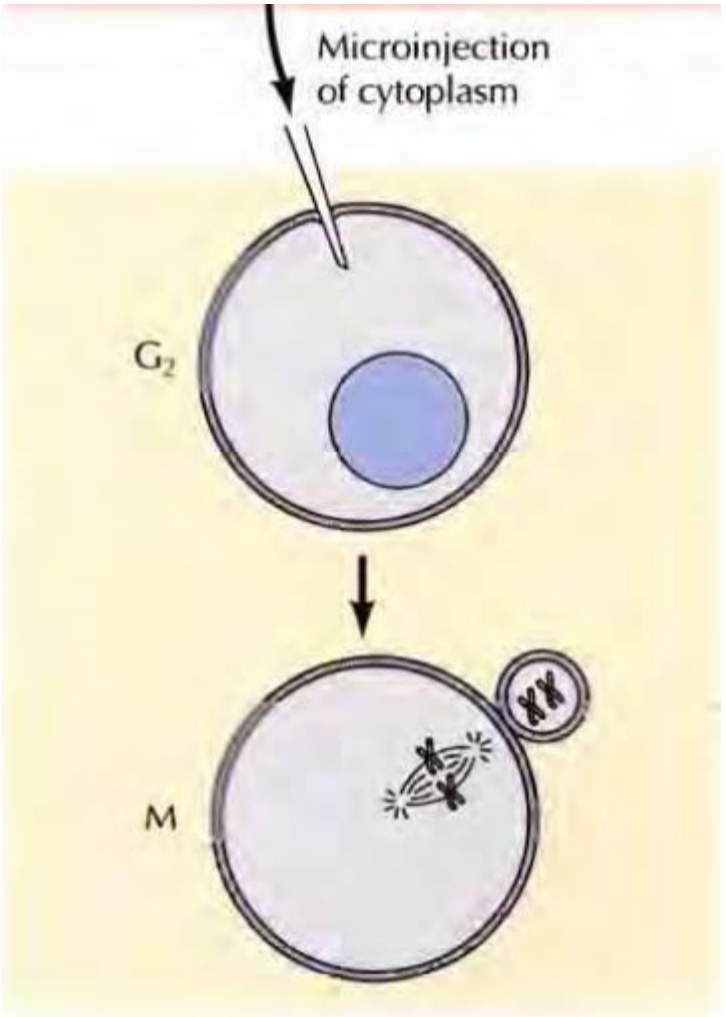
Role of checkpoints:

- **G1 checkpoint** allows **DNA repair** of the damage to take place before the cell enters S phase, where the damaged DNA would be replicated.
- **S-phase checkpoint** provides continual monitoring of the integrity of DNA to ensure that damaged DNA is repaired before it is replicated.
- **S-phase checkpoint** provides a quality control monitor to promote the repair of any errors that occur during DNA replication, such as the incorporation of incorrect bases or incomplete replication of segments of DNA.
- **G2 checkpoint** senses unreplicated DNA, which generates a signal that leads to cell cycle arrest. therefore prevents the initiation of M phase before completion of S phase, so cells remain in G2 until the genome has been completely replicated.
- **Spindle assembly checkpoint** monitors the alignment of chromosomes on the mitotic spindle, thus ensuring that a complete set of chromosomes is distributed accurately to the daughter cells.

Control of the Cell Cycle (Identification of MPF)

- In 1970, a series of cell fusion experiments carried out by Potu Rao and Robert Johnson of the University of Colorado helped open the door to understanding how the cell cycle is regulated.
- They wanted to know whether the cytoplasm of cells contains **regulatory factors** that affect cell cycle activities.
- They approached the question by fusing mammalian cells that were in different stages of the cell cycle
- The results of these experiments suggested that the ***cytoplasm of a mitotic cell contains diffusible factors that could induce mitosis in a nonmitotic cell***


G2
Progesterone
M
Microinjection
of cytoplasm


Microinjection
of cytoplasm
G2
M

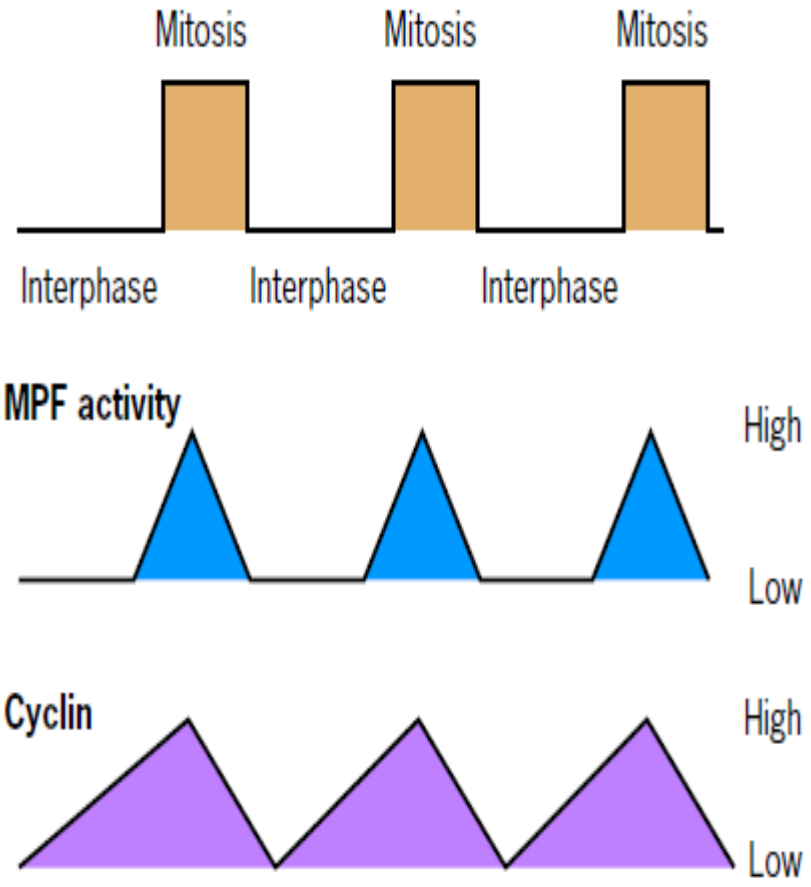
The Role of Protein Kinases

- entry of a cell into M phase is initiated by a protein called *maturation promoting factor (MPF).*
- MPF consists of two subunits:

  (1) a catalytic subunit *with* ***kinase*** activity that transfers **phosphate** groups from ATP to specific serine and threonine residues of specific protein substrates

  (2) a regulatory subunit called ***cyclin***

The term ***cyclin*** *was coined because the* *concentration of this regulatory* protein rises and falls in a predictable pattern with each cell cycle



Fluctuation of cyclin and MPF levels during the cell cycle.

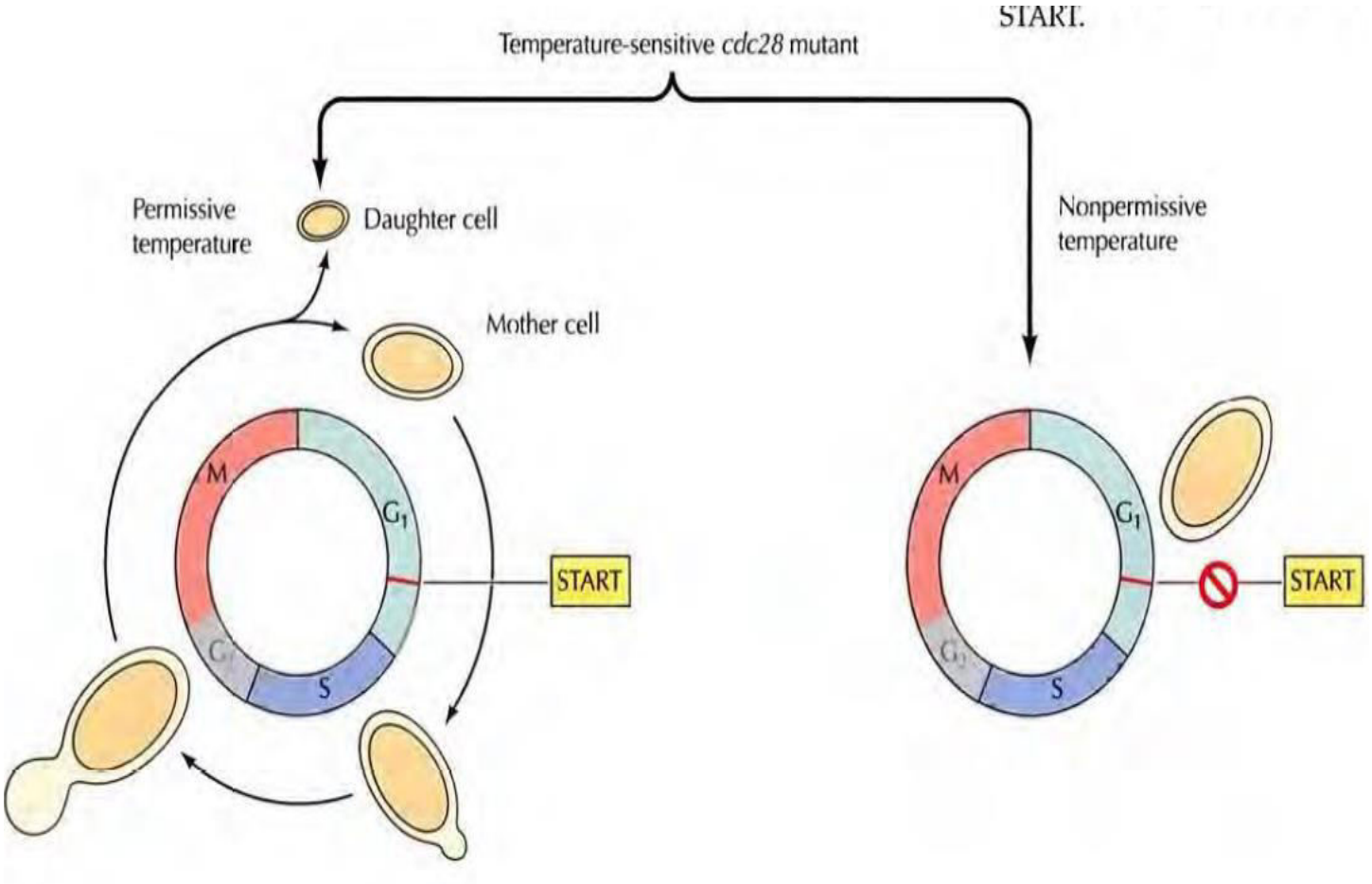
- results suggested that

  (1) progression of cells into mitosis depends on an enzyme whose sole activity is to ***phosphorylate*** other proteins, and

  (2) the activity of this enzyme is controlled by a subunit whose concentration varies from one stage of the cell cycle to another

MPF-like enzymes, are called **cyclin-dependent kinases (Cdks).**

- In 1970s, laboratories identified a gene that, when mutated, would cause the growth of cells at elevated temperature to stop at certain points in the cell cycle.
- The product of this gene, which was called *cdc2 (cdc* for cell division cycle mutants)in fission yeast and *CDC28* in budding yeast, was eventually found to be homologous to the catalytic subunit of MPF. it was a cyclin-dependent kinase (cdk)



Properties of *S. cerevisiae cdc28 mutants*

The temperature-sensitive *cdc28 mutant* replicates normally at the permissive temperature. At the nonpermissive temperature, however, progression through the cell cycle is blocked at START.

- The protein kinase encoded by the yeast *cdc2 and cdc28* genes has since been shown to be a conserved **cell cycle regulator** in all eukaryotes, which is known as Cdk1

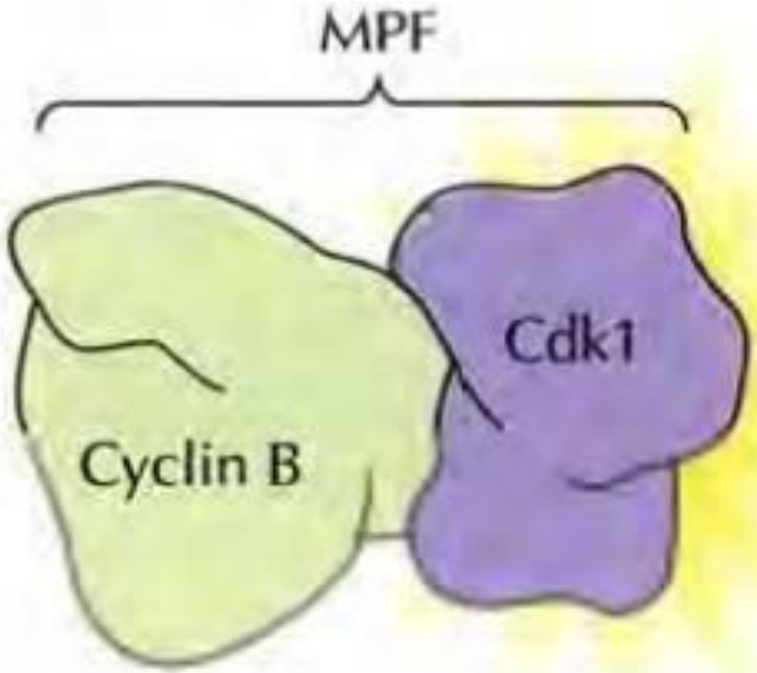
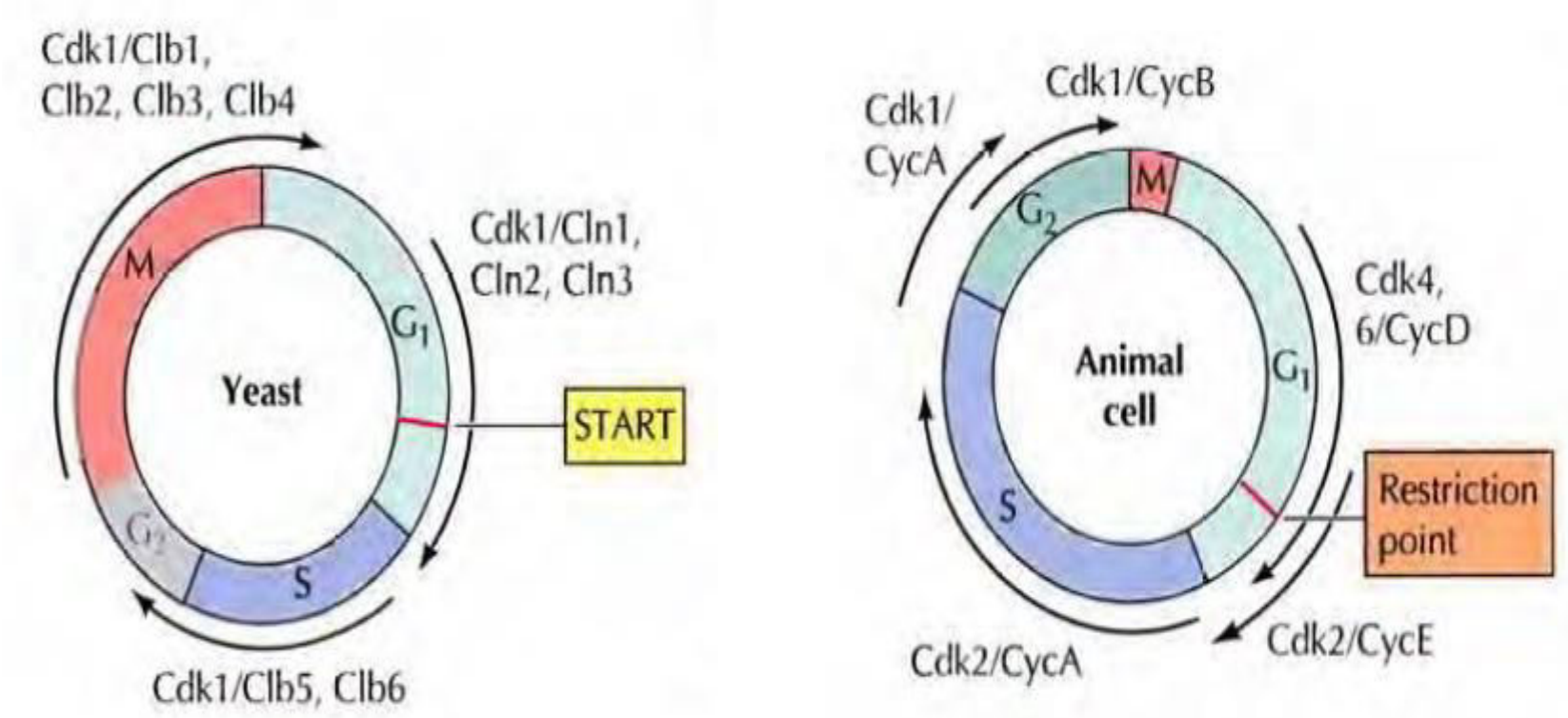


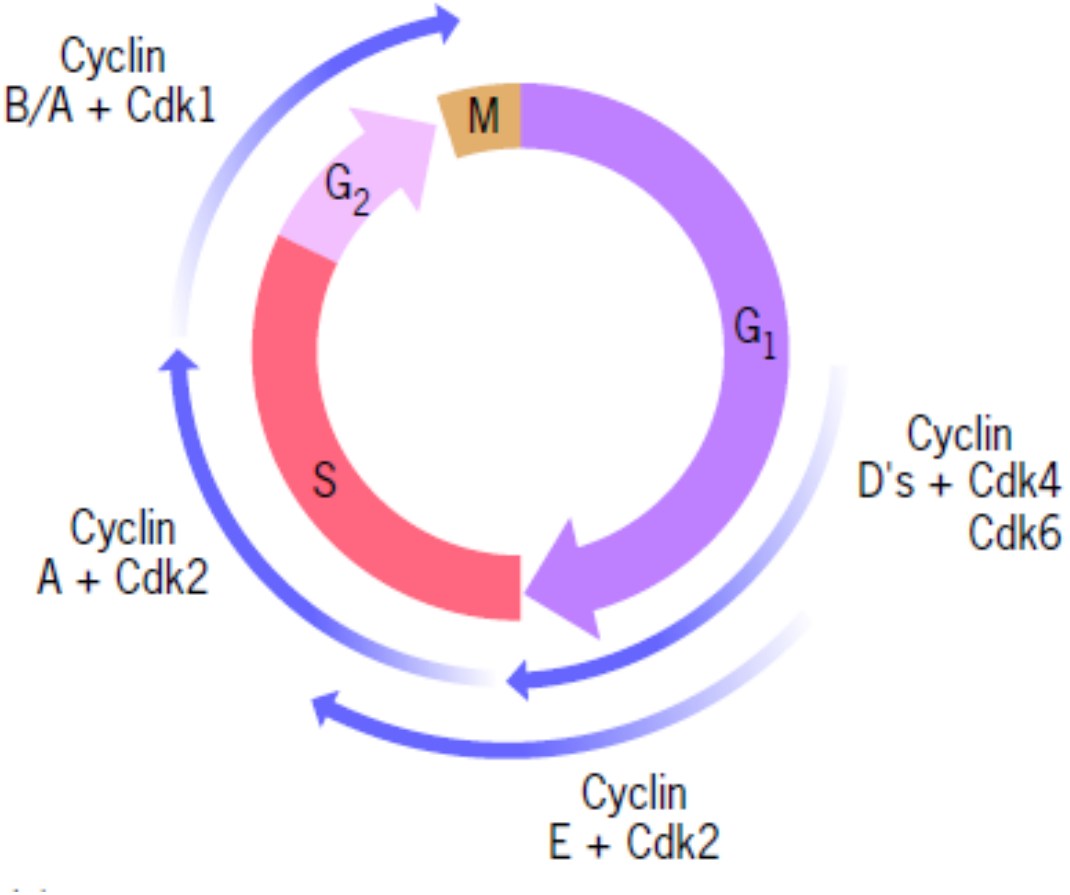
FIGURE 16.13 Structure of MPF
MPF is a dimer consisting of cyclin B and the Cdk1 protein kinase.

Complexes of cyclins and CDKs:

In yeast & In animal cells

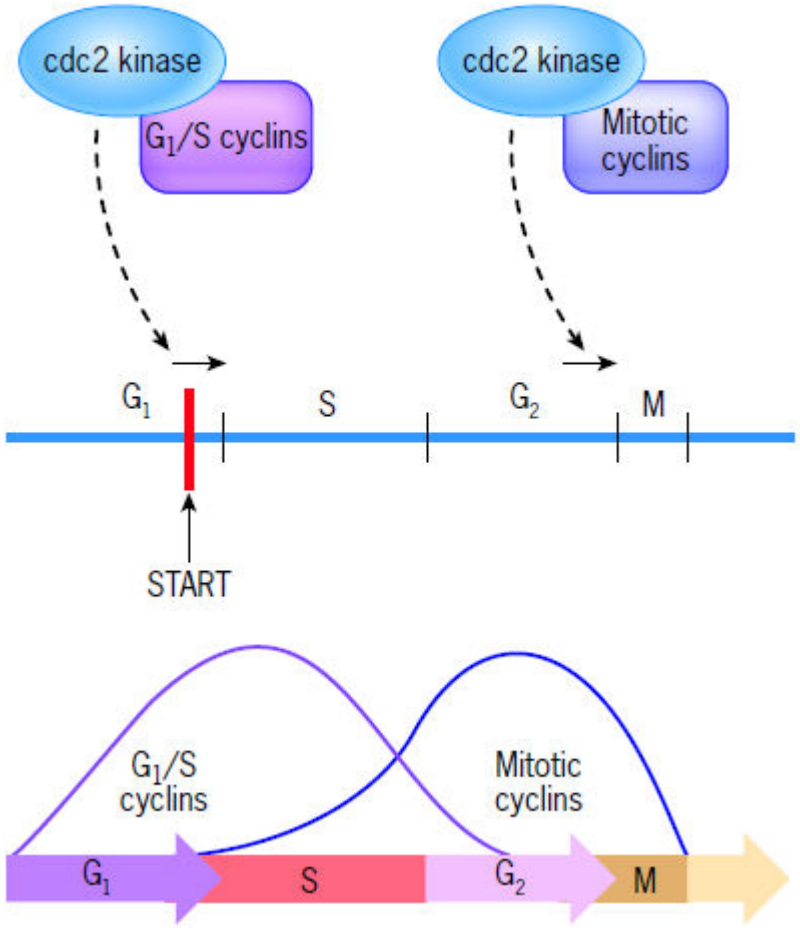


Cyclin-Cdks in the mammalian cell cycle

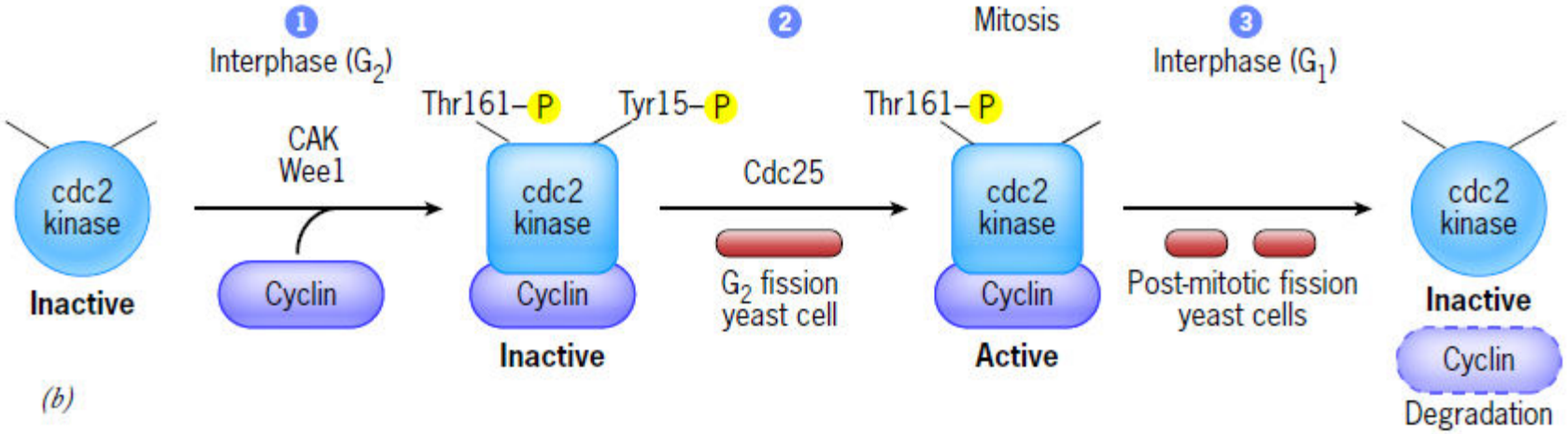


IN YEASTS

- The G2 to M transition is driven by Cdkl in association with the mitotic B-type cyclins (Clbl, Clb2, Clb3, and Clb4).
- Passage through START, however, is controlled by Cdkl in association with a distinct class of cyclins called G1 cyclins or Cln's
- Cdkl then associates with different B-type cyclins (Clb5 and Clb6), which are required for progression through S phase.



A simplified model for cell cycle regulation in fission yeast.
The cell cycle is controlled primarily at two points, **START** and the **G2–M transition**. Passage of a cell through these two critical junctures (black arrows) requires the activation of the same cdc2 kinase by different classes of cyclins, either G1/S or mitotic cyclins. A third major transition occurs at the end of mitosis and is triggered by a rapid drop in concentration of mitotic cyclins. (*Note: cdc*2 is also known as Cdk1.)



During G2 (In fission yeast cell cycle)

Step 1- the **cdc2 kinase** interacts with a **mitotic cyclin** but remains **inactive** as the result of **phosphorylation** of a key **tyrosine residue** (**Tyr 15** in fission yeast) by **Wee1.** A separate kinase, called **CAK** (Cdk-activating kinase), transfers a **phosphate** to another residue (**Thr 161**), which is required for **cdc2 kinase activity** later in the cell cycle.

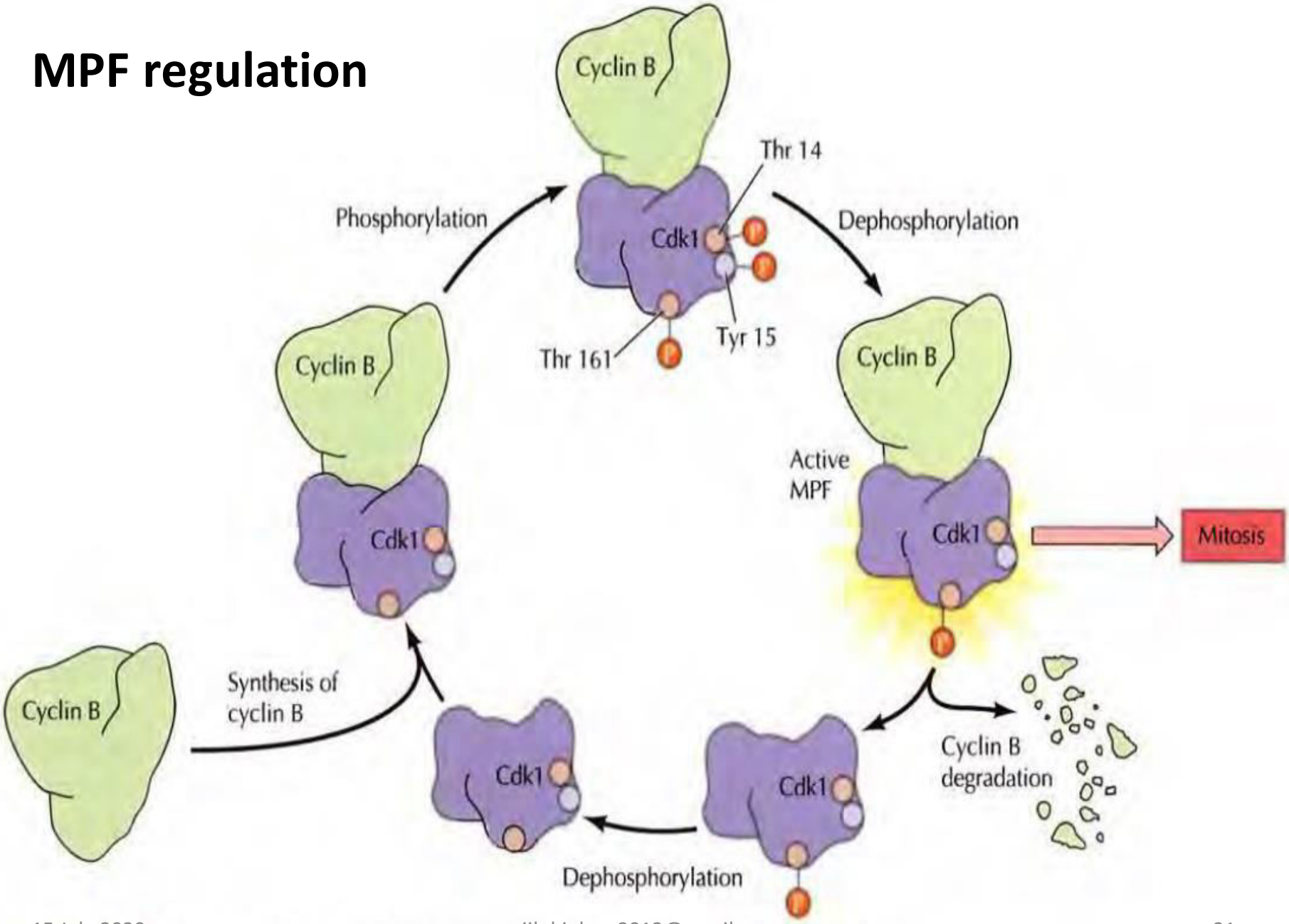
Step 2- When the cell reaches a critical size, an enzyme called **Cdc25 phosphatase** is activated, which removes the inhibitory phosphate on the **Tyr 15** residue. The resulting activation of the cdc2 kinase drives the cell into mitosis.

Step 3 - By the end of mitosis the stimulatory phosphate group is removed from **Thr 161** by another **phosphatase**. The free cyclin is subsequently degraded, and the cell begins another cycle.

In mammalian cells

- Cyclin B is synthesized and forms complexes with Cdkl during G2.
- As these complexes form, Cdkl is **phosphorylated** at two critical regulatory positions.
- One of these phosphorylations occurs on **threonine-161** and is required for **Cdk1 kinase activity**.
- The second is a phosphorylation of **tyrosine-15** and of the adjacent **threonine-14** in vertebrates. Phosphorylation of **tyrosine-I5**, catalyzed by a protein kinase called **Wee1**, inhibits Cdk1 activity and leads to the accumulation of inactive **Cdkl/cyclin B** complexes throughout G2.
- The transition from G2 to M is then brought about by activation of the Cdk1/cyclin B complex as a result of **dephosphorylation** of **threonine-14** and **tyrosine-15** by a protein **phosphatase** called **Cdc25C**.

MPF regulation



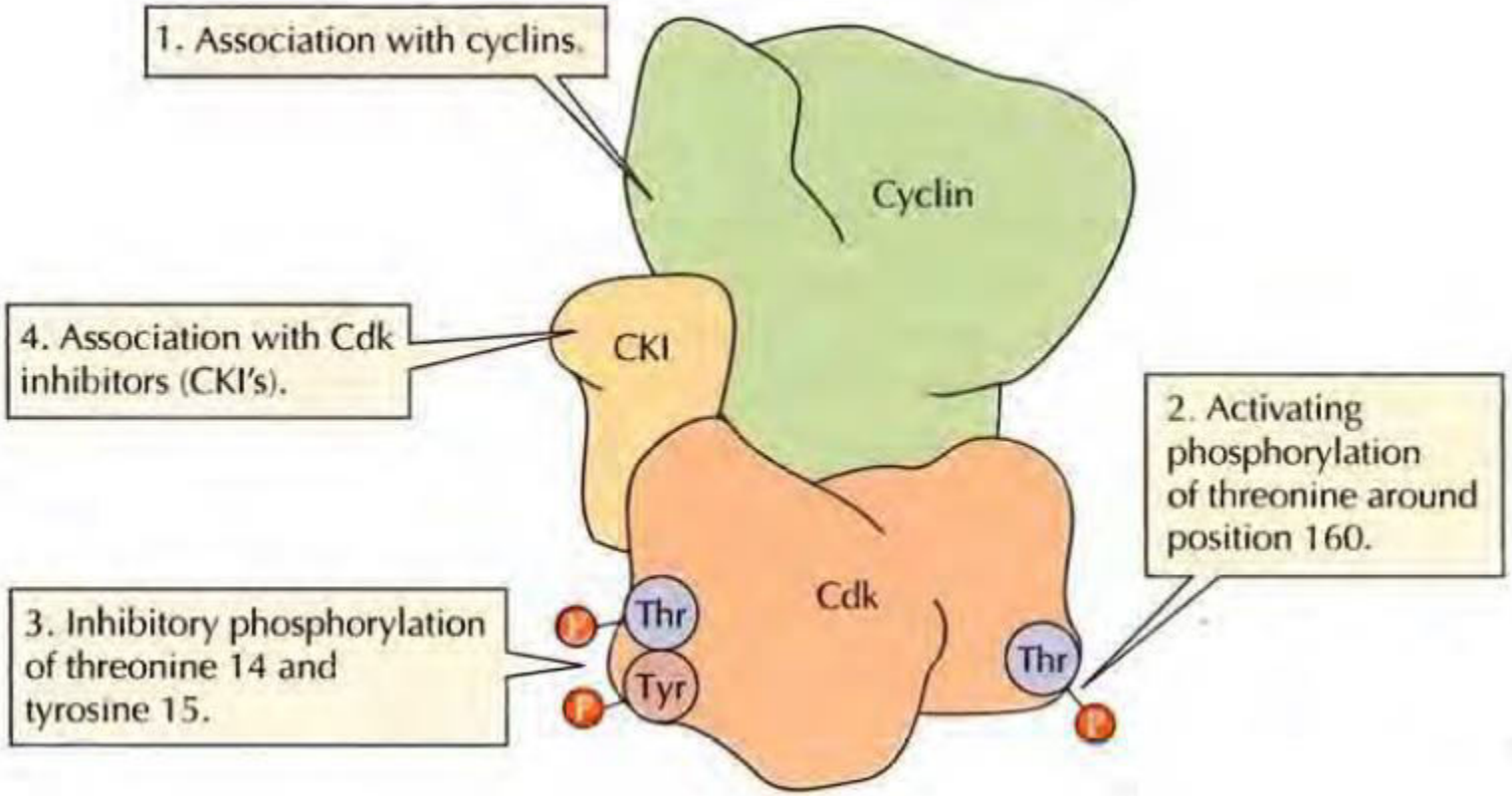
Regulation of activity of Cdk's during cell cycle progression :

1. **association** of Cdk's **with their cyclin** partners, formation of specific Cdk/ cyclin complexes is controlled by **cyclin synthesis** and **degradation**.
2. activation of Cdk/ cyclin complexes requires **phosphorylation** of a conserved Cdk threonine residue around position 160. This activating phosphorylation of the Cdk's is catalyzed by an enzyme called CAK (for Cdk-activating kinase), which is itself composed of a Cdk (Cdk7) complexed with **cyclin H**

3. the third mechanism of Cdk regulation involves **inhibitory phosphorylation of tyrosine residues** near the Cdk amino terminus, catalyzed by the **Weel** protein kinase. In particular, both Cdkl and Cdk2 are inhibited by phosphorylation of tyrosine-I5, and the adjacent threonine-14 in vertebrates.
4. also controlled by the binding of inhibitory proteins called **Cdk inhibitors or CKIs**

Mechanisms of Cdk regulation

The activities of Cdk's are regulated by four molecular mechanisms



CKI:

- In mammalian cells, two families of Cdk inhibitors are responsible for regulating different Cdk's
- 1. Members of the Ink4 family specifically bind to and inhibit monomeric Cdk4 and Cdk6, so the lnk4 CKis act to inhibit progression through G1
- 2. members of the Cip/Kip family bind to and inhibit the protein kinase activity of both Cdkl and thereby, inhibiting progression through all phases of the cell cycle.

In budding yeast

- for example,
- a protein called Sic1 acts as a Cdk inhibitor during G1.
- The degradation of Sic1 allows the cyclin–Cdk that is present in the cell to initiate DNA replication

TABLE 16.1 Cdk Inhibitors

Inhibitor	Cdk or Cdk/ cyclin complex	Cell cycle phase affected
Ink4 family (p15, p16, p18, p19)	Cdk4 and Cdk6	G_1
Cip/Kip family (p21, p27, p57)	Cdk1/cyclin A	G_2
	Cdk1/cyclin B	G_2/M
	Cdk2/cyclin A	S
	Cdk2/cyclin E	G_1

Growth Factors and the Regulation of G1 Cdk's

- **Cyclin Dl synthesis** is induced in response to **growth factor** stimulation in part as a result of signaling through the Ras/Raf/MEK/ERK pathway, and cyclin D1 continues to be synthesized as long as growth factors are present.
- cyclin D1 is also rapidly degraded, so its intracellular concentration rapidly falls if growth factors are removed. Thus, as long as growth factors are present through G1, complexes of Cdk4, 6 / cyclin D1 drive cells through the restriction point.

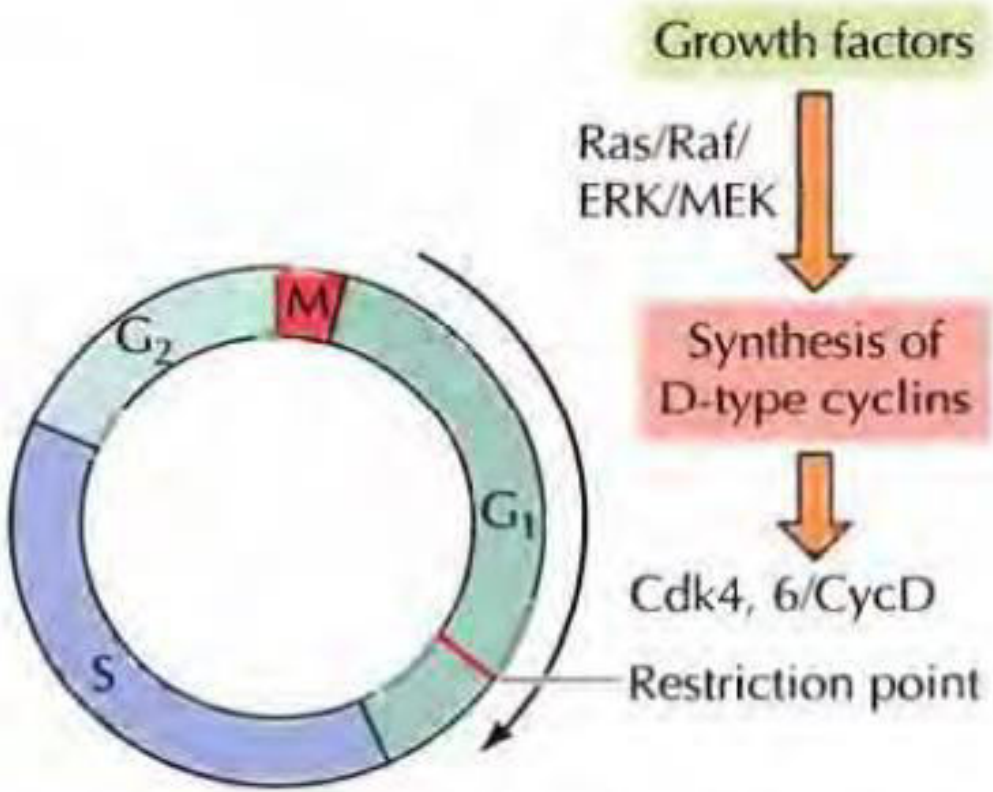
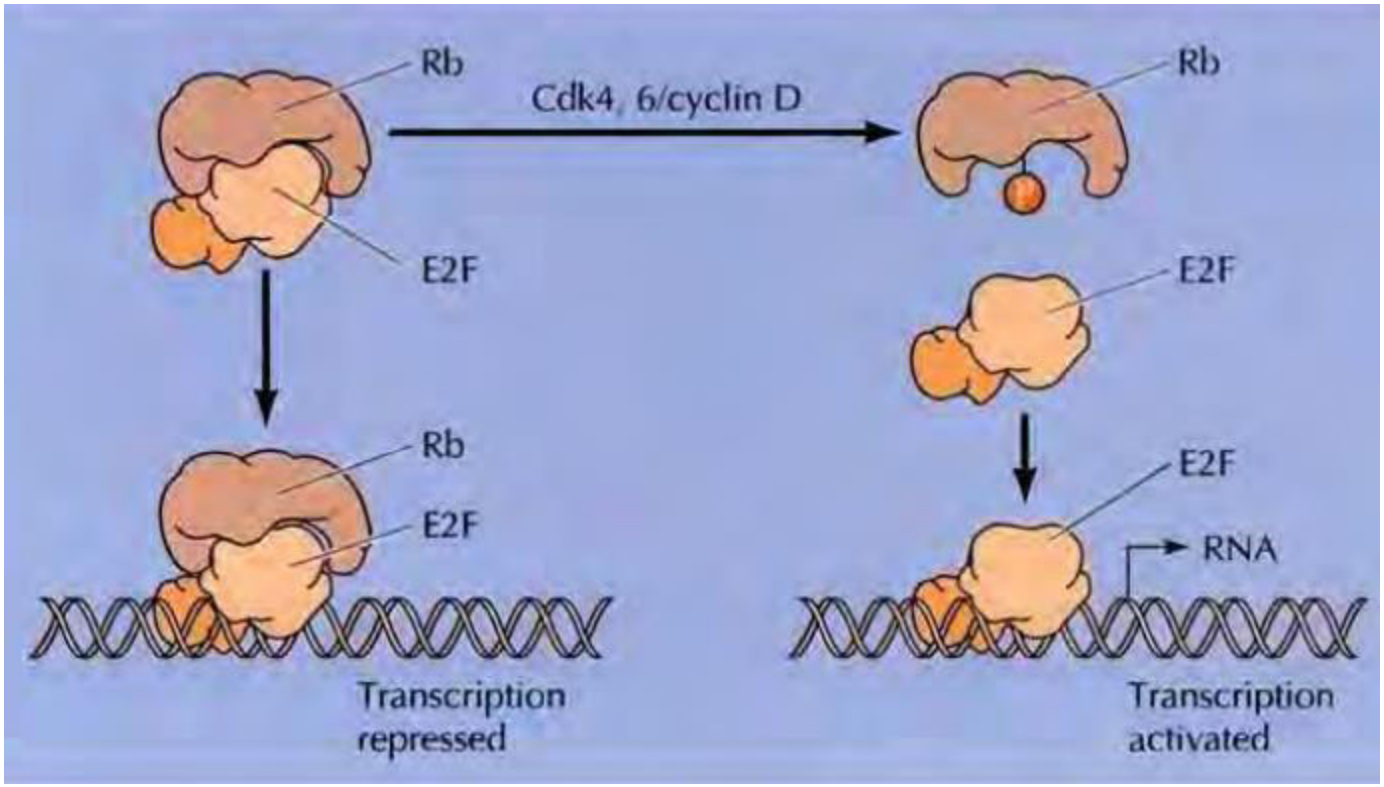


FIGURE 16.17 Induction of D-type cyclins Growth factors regulate cell cycle progression through the G_1 restriction point by inducing synthesis of D-type cyclins via the Ras/Raf/MEK/ERK signaling pathway.

MUTATIONS

- Resulting in continual unregulated expression of cyclin D1 contribute to the development of a variety of human cancers, including lymphomas and breast cancers.
- Similarly, mutations that inactivate the Ink4 Cdk inhibitors that bind to Cdk4 and Cdk6 are commonly found in human cancer cells

CONNECTION between cyclin D, growth control, and cancer



Cell cycle regulation of Rb and E2F

Rb -tumor suppressor gene a target of Cdk4, 6/cyclin D complexes

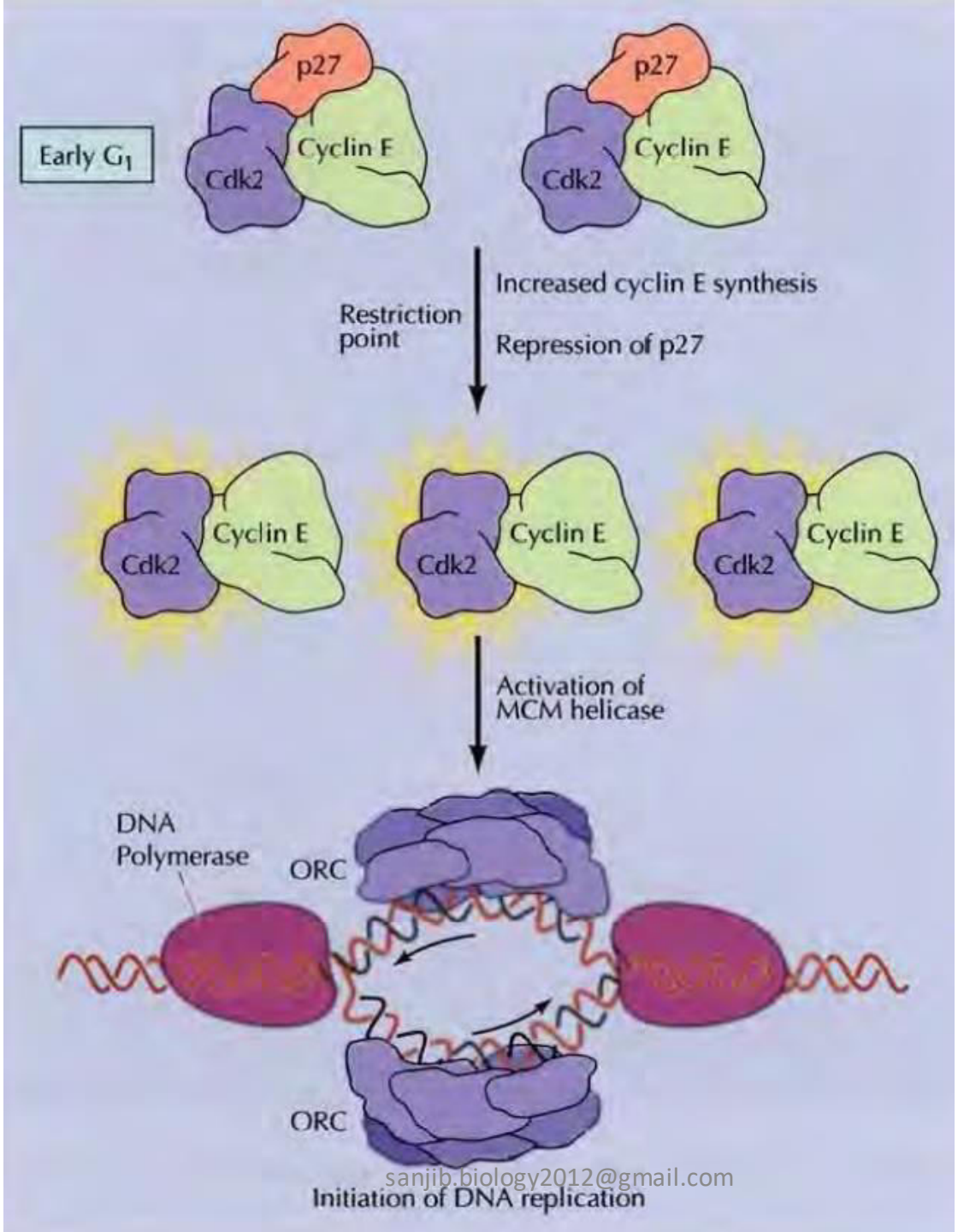
- In its under phosphorylated form (present in G0 or early G1), Rb binds to members of the E2F family of transcription factors, which regulate expression of several genes involved in cell cycle progression, including the gene encoding **cyclin E**.
- E2F binds to its target sequences in either the presence or absence of Rb.

- **Rb acts as a repressor**, so the Rb /E2F complex suppresses transcription of E2F-regulated genes.
- **Phosphorylation of Rb** by Cdk4, 6/ cyclin D complexes results in its dissociation from E2F, which then activates transcription of its target genes.

Cdk2/ cyclin E and entry into S phase

- activity of Cdk2/cyclin E is inhibited in G0 or early G1 by the Cdk inhibitor **p27**, which belongs to the Cip/Kip family.
- In addition, growth factor signaling via both the Ras/Raf/MEK/ERK and PI 3-kinase/ Akt pathways reduces the transcription and translation of p27, lowering the levels of p27 within the cell.
- Passage through the restriction point induces the synthesis of cyclin E via activation of E2F.

- **Increased synthesis of cyclin D** leads to the **binding of p27 to Cdk4, 6/ cyclin D complexes**, sequestering it from binding to Cdk2/cyclin E.
- Once Cdk2 becomes activated, it brings about the complete **degradation of p27** by **phosphorylating** it and targeting it for ubiquitination.
- Cdk2/ cyclin E complexes then initiate S phase by activating the **MCM helicase** proteins at replication origins leading to the initiation of DNA synthesis

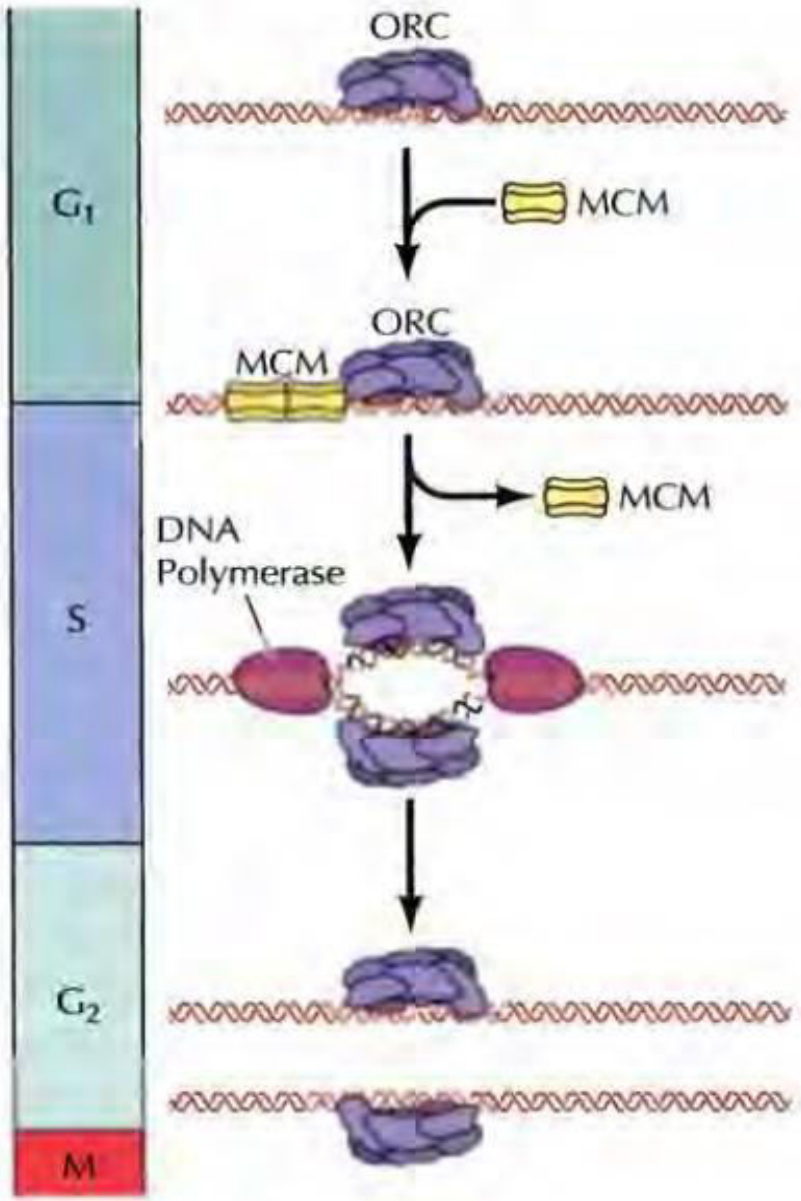


Restriction of DNA replication

DNA replication is restricted to once per cell cycle by the **MCM helicase** proteins that bind to origins of replication together with **ORC** (origin recognition complex) **proteins** and are required for the initiation of DNA replication.

MCM proteins are only able to **bind to DNA in G1** allowing DNA replication to initiate in S phase.

Once initiation has occurred, the MCM proteins are displaced so that replication cannot initiate



The Licensing of DNA Replication in Eukaryotes

- How does a cell ensure that replication is initiated at thousands of origins only once per cell cycle?

The Licensing of DNA Replication in Eukaryotes

- The precise replication of DNA is accomplished by the separation of the initiation of replication into two distinct steps.
- In the **first step**, the origins are licensed, meaning that they are approved for replication. This step is early in the cell cycle when a **replication licensing factor attaches to an origin.**
- In the **second step**, the replication machinery initiates replication at each licensed origin. The key is that the replication machinery functions only at licensed origins.
- As the replication forks move away from the origin, the licensing factor is removed, **leaving the origin in an unlicensed state**, where replication cannot be initiated again until the license is renewed.
- To ensure that replication takes place only once per cell cycle, the licensing factor is active only after the cell has completed mitosis and before the replication is initiated.

Mechanism:

- The eukaryotic *licensing factor* is a complex called **MCM** (for **minichromosome maintenance**), which contains a **DNA helicase** that unwinds a short stretch of DNA in the initiation of replication.
- MCM must bind to the DNA for replication to initiate at an origin.
- After replication has begun at an origin, a protein called **Geminin** prevents **MCM** from binding to DNA and reinitiating replication at that origin.
- At the end of mitosis, Geminin is degraded, allowing **MCM** to bind once again to DNA and relicense the origin.

DNA Damage Checkpoints

- operative in G1, S, and G2 phases of the cell cycle, serve to halt cell cycle progression and allow time for the damage to be repaired before DNA replication or cell division proceeds

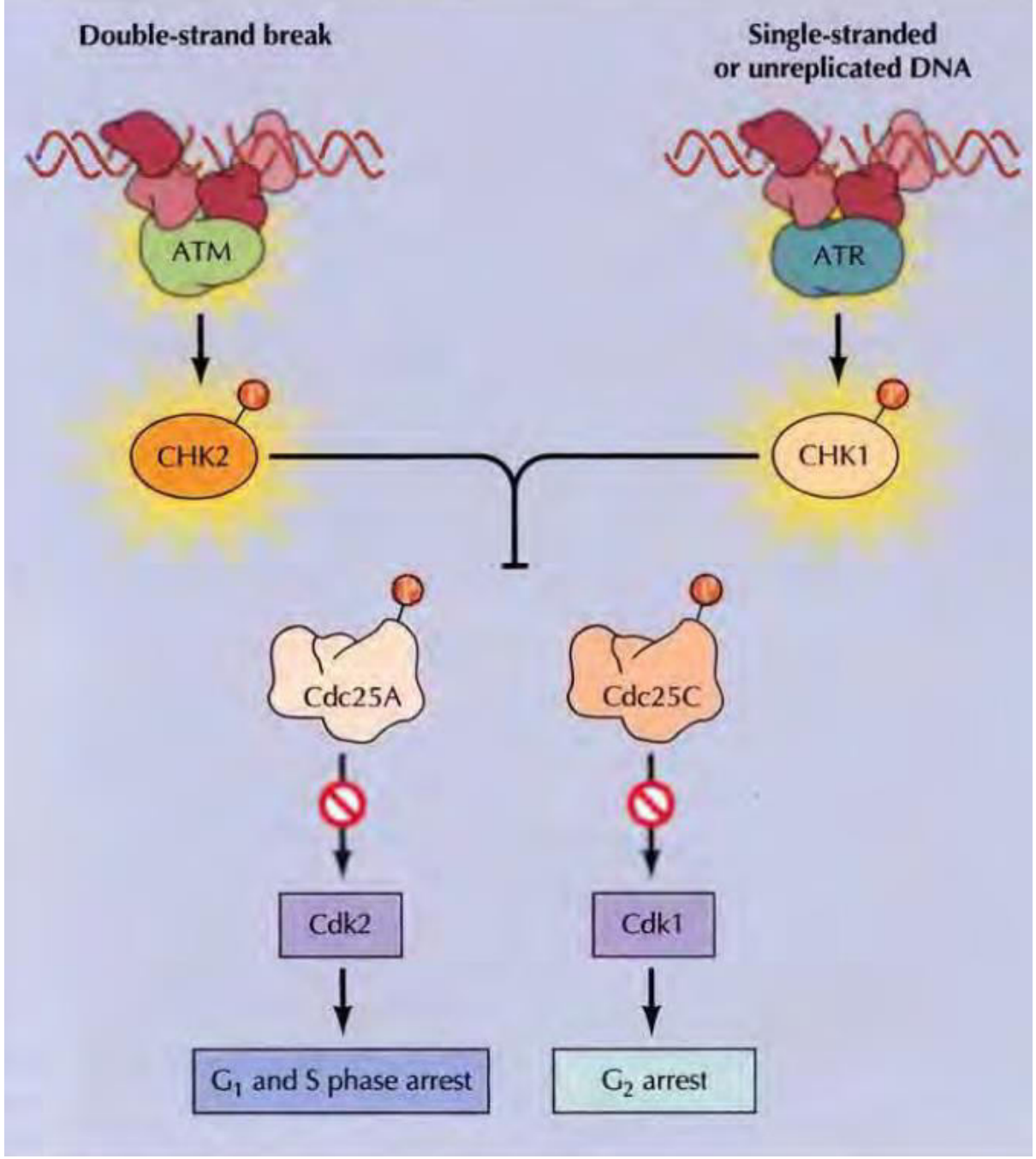
Role of ATR & ATM

- Cell cycle arrest at the DNA damage checkpoints is initiated by the **ATM** or **ATR protein kinases**, which **recognize damaged** or **unreplicated DNA**
- ATM (gene responsible for ataxia-telangiectasia) is activated principally by double-strand breaks, while ATR is activated by single stranded breaks or unreplicated DNA.
- Once activated by DNA damage, ATM and ATR **phosphorylate** and **activate** the checkpoint kinases **CHK2** and **CHK1**, respectively, bringing about cell cycle arrest

- Both CHK1 and CHK2 **phosphorylate** and **inhibit Cdc25C phosphatases**, which are required to activate Cdk/ cyclin complexes by removing inhibitory phosphorylations

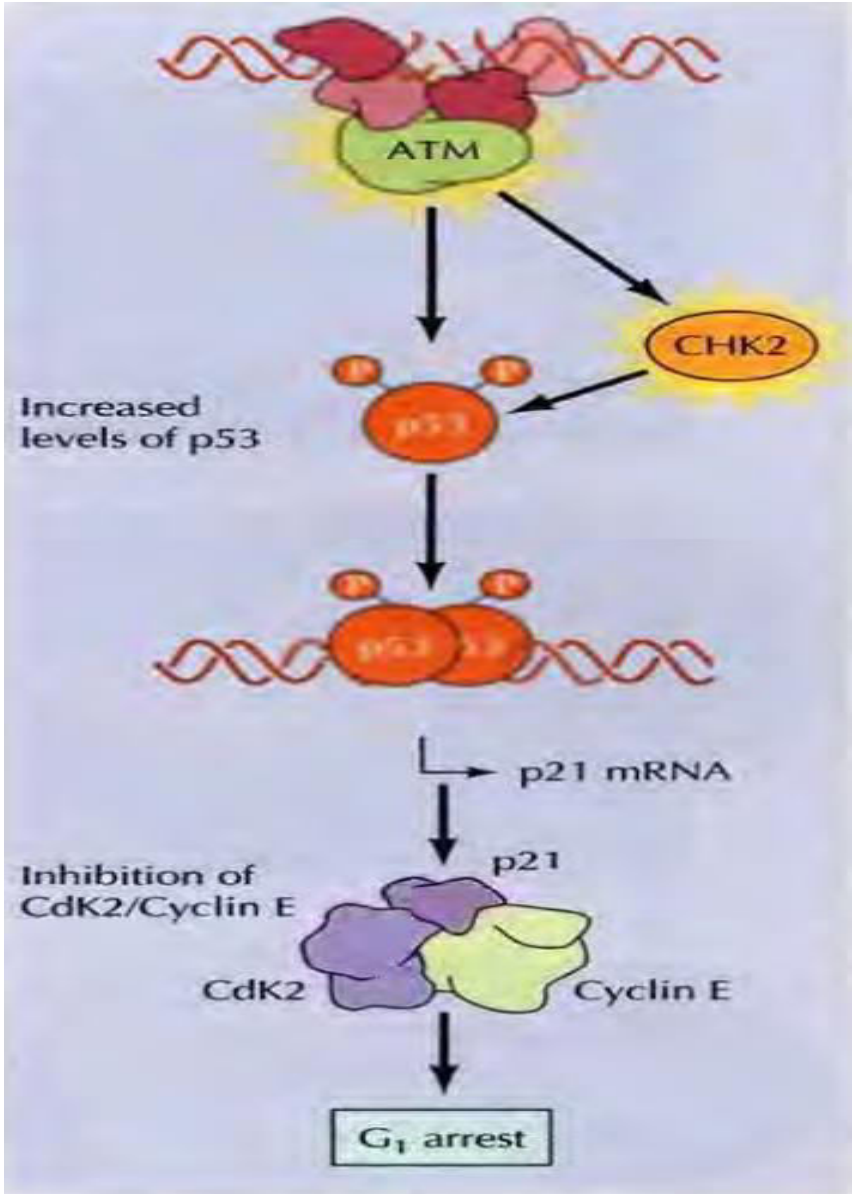
- At the **G1** and **S phase** checkpoints, CHK1 and CHK2 **phosphorylate Cdc25A**, which is required to activate complexes of Cdk2 and cyclins A or E.

- **Phosphorylation** leads to the rapid **degradation of Cdc25A**, resulting in inhibition of Cdk2
- At the G2 checkpoint, CHK1 and CHK2 **phosphorylate** and **inhibit Cdc25C**, which is responsible for activating Cdk1 / cyclin B complexes.
- In the absence of Cdk1 activation, progression to mitosis is blocked and the cell remains arrested in G2.

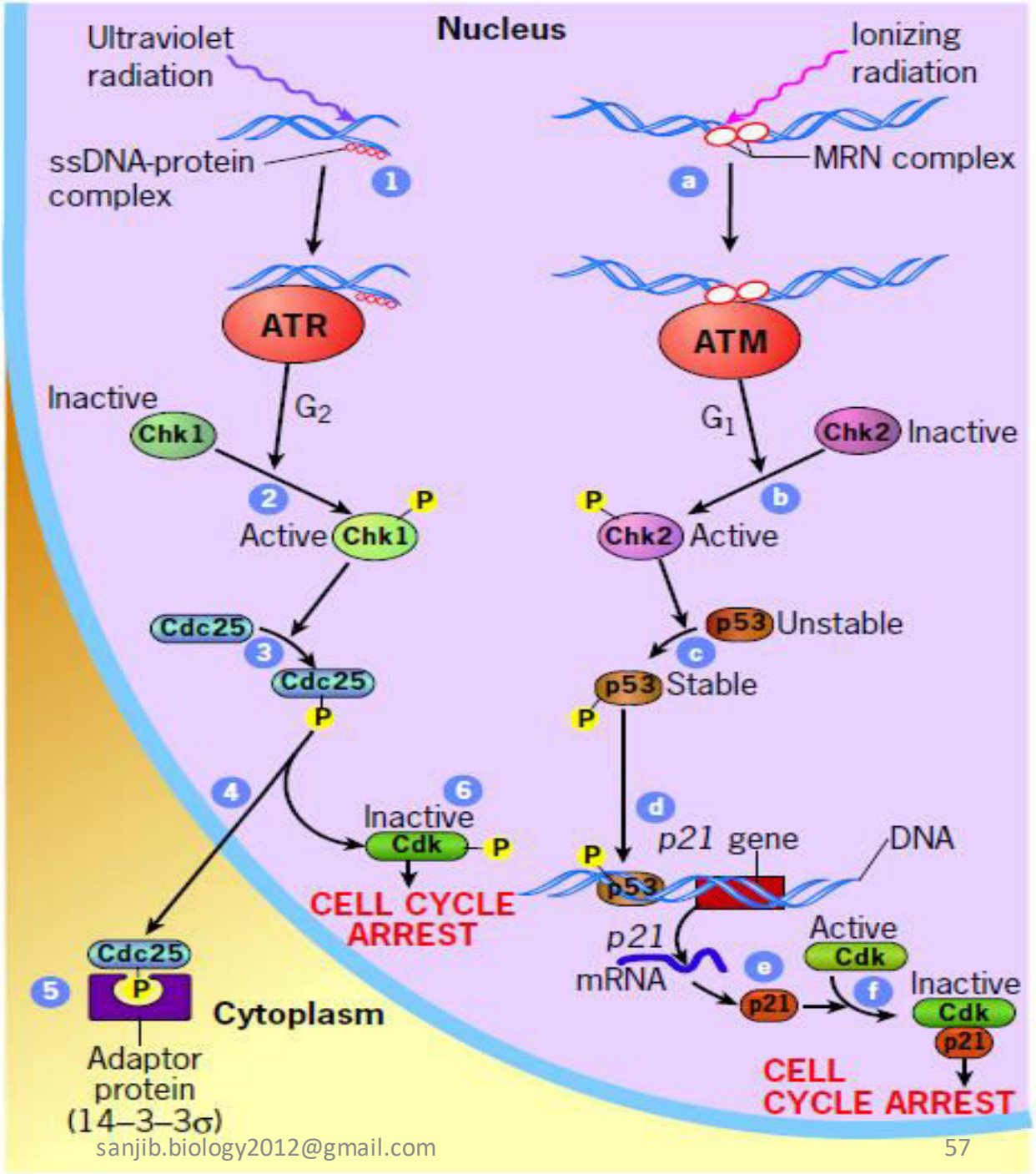

Double-strand break
Single-stranded
or unreplicated DNA
ATM
ATR
CHK2
CHK1
Cdc25A
Cdc25C
Cdk2
Cdk1
G1 and S phase arrest
G2 arrest

Role of p53 in G1 arrest

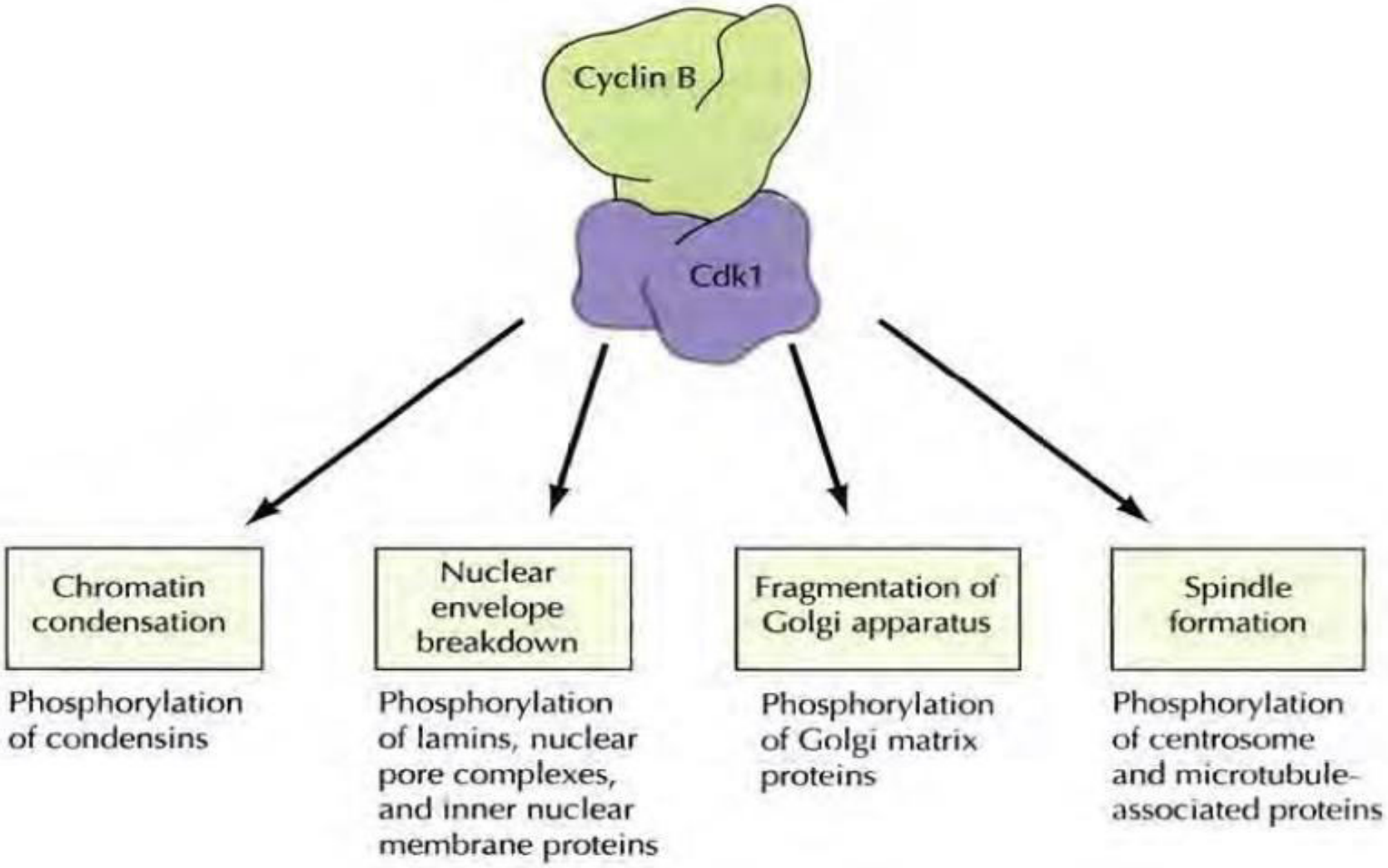
- In mammalian cells, arrest at the G1 checkpoint is also mediated by the action of an additional protein known as **p53**, which is phosphorylated by both **ATM** and **CHK2**
- **Phosphorylation stabilizes p53**, which is otherwise rapidly degraded, resulting in a rapid increase in p53 levels in response to damaged DNA.
- The **p53 protein** is a **transcription factor**, and its increased expression leads to the induction of the **Cip/Kip family Cdk inhibitor - p21**.
- The p21 protein inhibits Cdk2/ cyclin E complexes, leading to cell cycle arrest in G1



Models for the mechanism of action of two DNA damage checkpoints

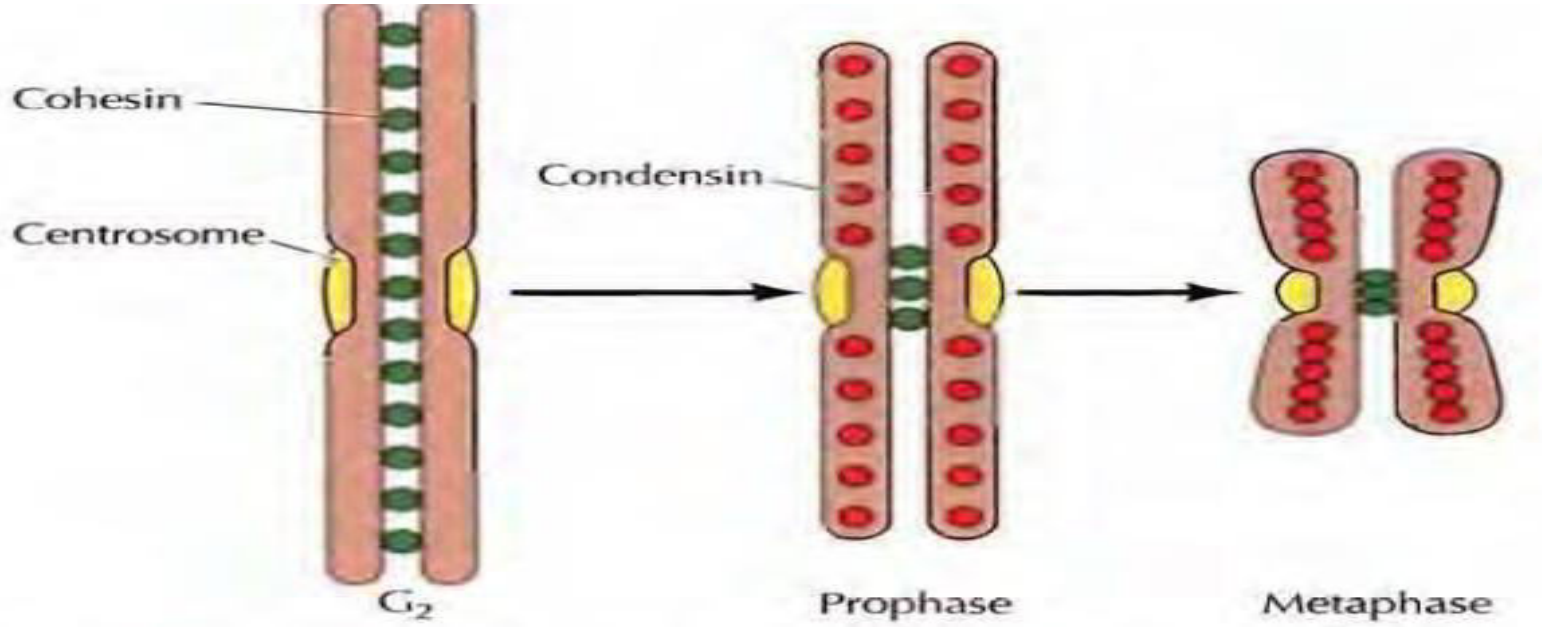


Cdkl/CyclinB and Progression to Metaphase



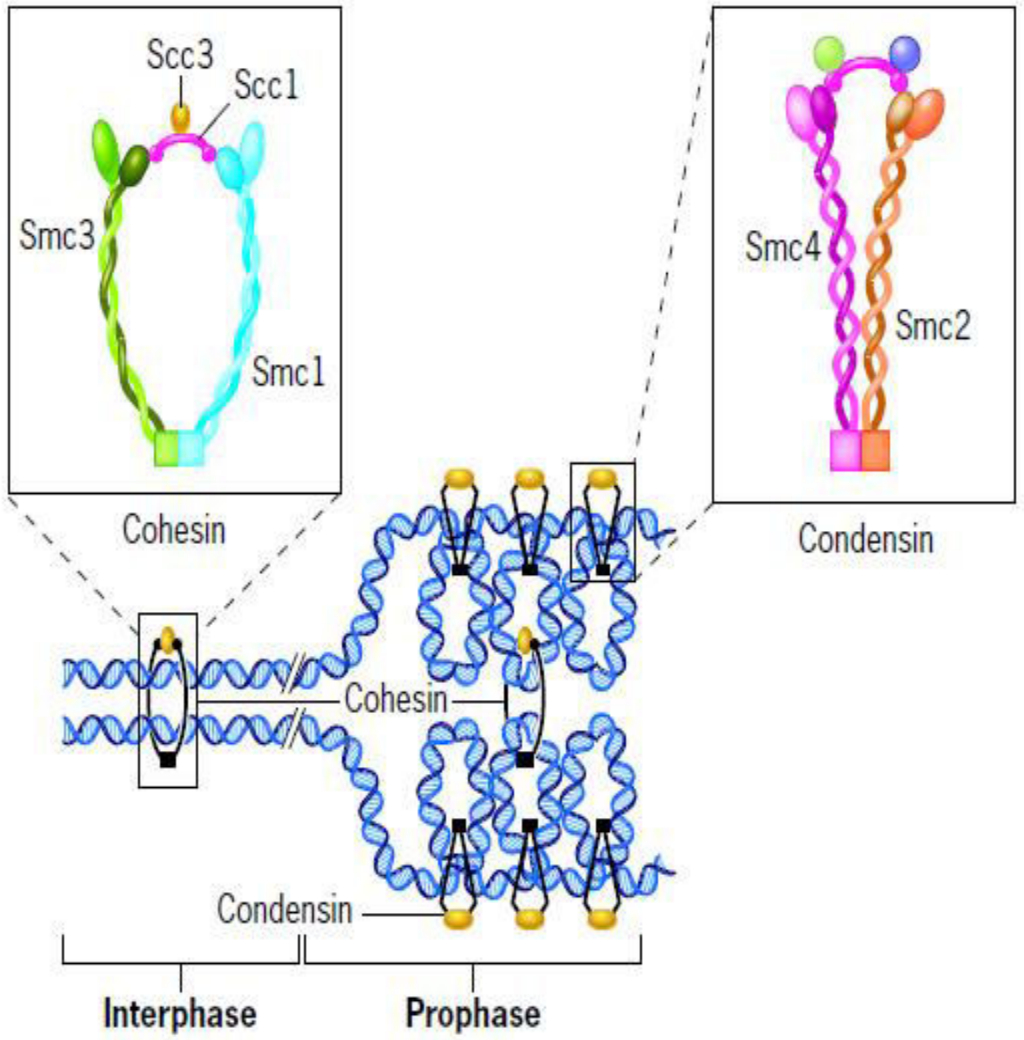
Condensins induce **chromatin condensation**

- Condensation of interphase chromatin to form the compact chromosomes of mitotic cells is a key event in mitosis
- It is driven by protein complexes called **condensins**, which are members of a class of "**structural maintenance of chromatin**"(SMC) **proteins**
- Both **condensins** and **cohesins**, contribute to chromosome segregation during mitosis
- **Cohesins** bind to **DNA** in **S phase** and maintain the linkage between sister chromatids following DNA replication
- As the cell enters M phase, the **condensins are activated** by **Cdkl/cyclin B phosphorylation**
- **Condensins** then **replace** the **cohesins** along most of the length of the chromosome, so that the sister chromatids remain linked only at the **centromere**
- The condensins also induce chromatin condensation, leading to the formation of metaphase chromosomes.



The action of cohesins and condensins

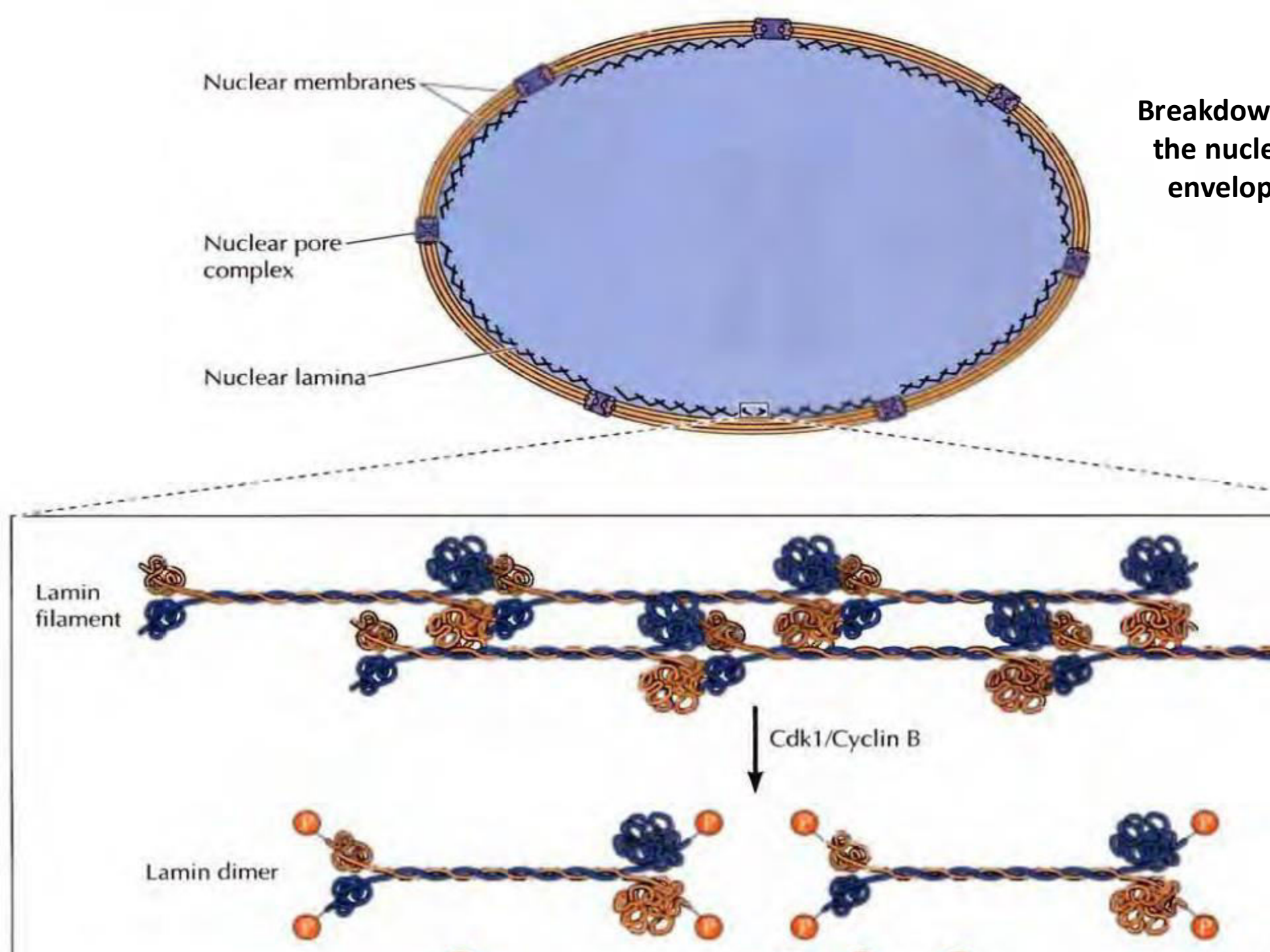
Cohesins bind to DNA during **S phase** and maintain the **linkage** between **sister chromatids** following DNA replication in S and G2. As the cell enters M phase. the cohesins are replace by condensins along most of th chromosome, remaining only at the centromere. Phosphorylation by Cdkl activates the condensins, which drive chromatin condensation.



Model for the roles of condensin and cohesin in the formation of mitotic chromosomes

Breakdown of the nuclear envelope

- It includes (1)nuclear membranes fragmentation, (2) nuclear pore complexes dissociation, and (3) the nuclear lamina depolymerization.
- **Depolymerization** of the nuclear lamina results from **phosphorylation** of the **lamins** by **Cdkl.** The **lamin filaments** are broken down into individual **lamin dimers**.
- Cdkl also phosphorylates **several proteins** in the **inner nuclear membrane** and the **nuclear pore complex**, leading to disassembly of nuclear pore complexes and detachment of the inner nuclear membrane from lamins and chromatin



Breakdow
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Fragmentation of Golgi apparatus

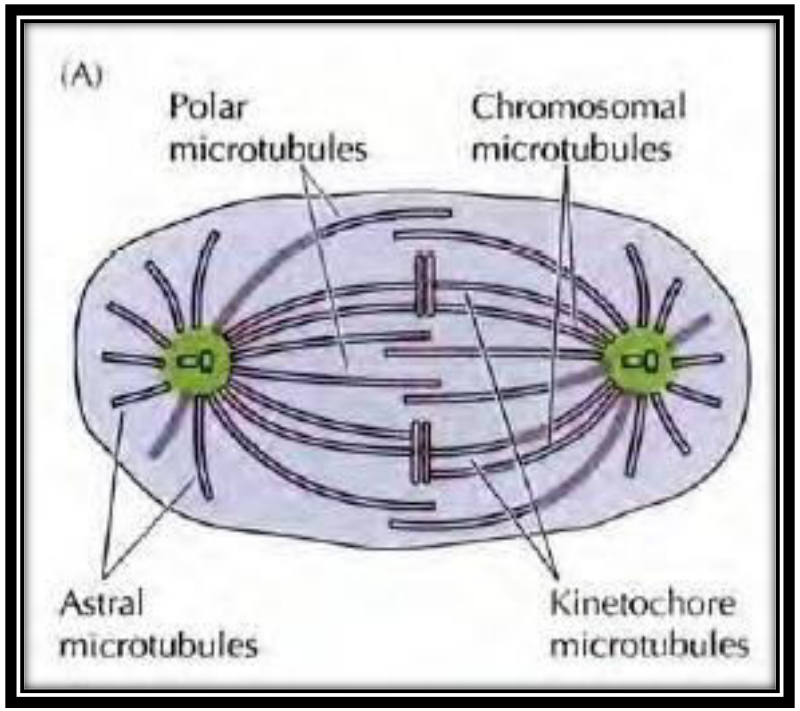
- The Golgi apparatus fragments into small vesicles at mitosis, which may either be absorbed into the endoplasmic reticulum or distributed directly to daughter cells at cytokinesis.
- Cdkl phosphorylate **Golgi matrix proteins** (such as **GM130** and **GRASP-65**), which are required for the docking of COPI-coated vesicles to the Golgi membrane.
- **Phosphorylation** by **Cdkl inhibits** vesicle **docking** and **fusion**, leading to **fragmentation of the Golgi apparatus**.

Spindle formation:

- At the beginning of prophase, activation of Cdkl leads to separation of the centrosomes, which were duplicated during S phase.
- The centrosome, then move to opposite sides of the nucleus and undergo a process of maturation during which they enlarge and recruit y-tubulin and other proteins needed for spindle assembly.
- Centrosome maturation and spindle assembly involves the activity of protein kinases of the **Aurora** and **Polo** like **kinase families**, which are located at the centrosome
- The rate of microtubule turnover increases five- to tenfold during mitosis, resulting in depolymerization and shrinkage of the interphase microtubules.
- This increased turnover is due to **phosphorylation** of **microtubule-associated proteins**, either by **Cdkl** or other mitotic protein kinases, such as the **Aurora** or **Polo-like kinases**

Spindle formation:

- The number of microtubules emanating from the centrosomes also increases, so the interphase microtubules are replaced by large numbers of short microtubules radiating from the centrosomes.
- The breakdown of the nuclear envelope then allows some of the spindle microtubules to attach to chromosomes at their kinetochores initiating the process of chromosome movement that characterizes prometaphase.
- Consequently, the chromosomes in prometaphase shuffle back and forth between the centrosomes and the center of the spindle
- Microtubules from opposite poles of the spindle eventually attach to the two kinetochores of sister chromatids (which are located on opposite sides of the chromosome), and the balance of forces acting on the chromosomes leads to their alignment on the metaphase plate in the center of the spindle



The metaphase spindle

Progression from Metaphase to Anaphase

The Spindle Assembly Checkpoint & APC

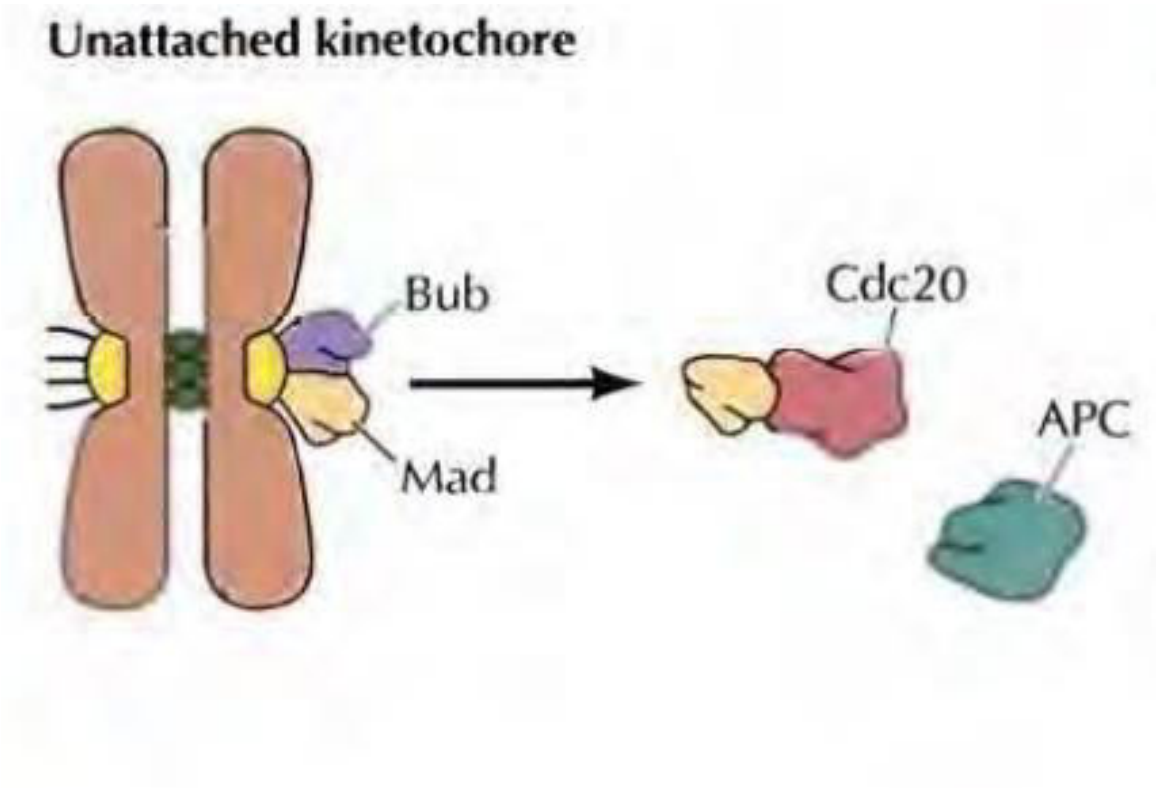
- **Spindle assembly checkpoint (Mad/Bub complex)** monitors the proper alignment of chromosomes on the metaphase spindle
- Once this has been accomplished, the cell proceeds to initiate anaphase and complete mitosis.

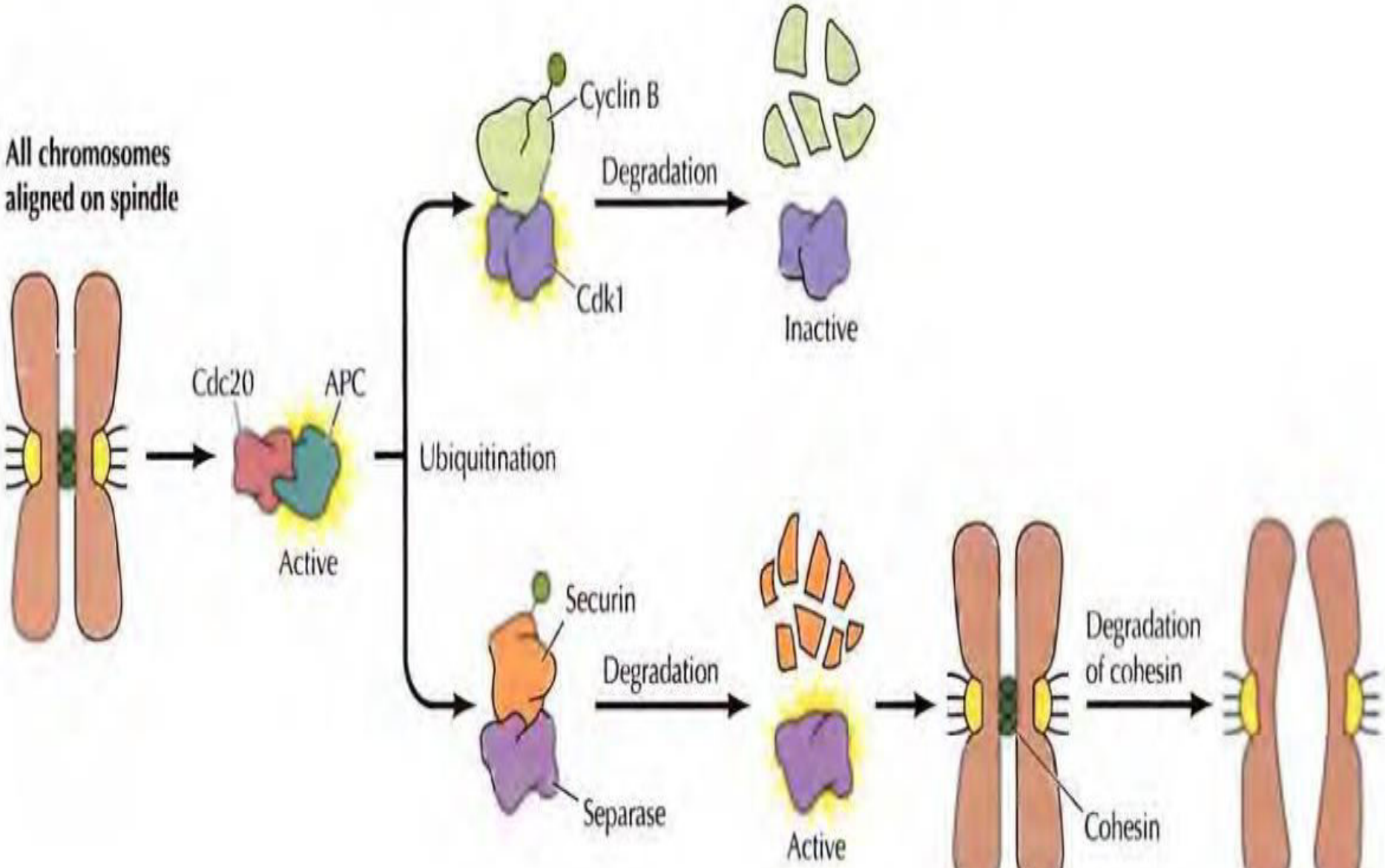
- The progression from metaphase to anaphase results from **ubiquitin-mediated proteolysis** of key regulatory proteins, triggered by activation of an **E3 ubiquitin ligase** called the **anaphase-promoting complex (APC)**
- Activation of the **APC** is induced at the beginning of mitosis, so the activation of Cdkl/ cyclin B ultimately triggers its own destruction.
- The **APC** remains inhibited, however, until the cell passes the **spindle assembly checkpoint**, after which activation of the ubiquitin degradation system brings about the transition from metaphase to anaphase and progression through the rest of mitosis

Role of Mad/Bub complex :

- The checkpoint is mediated by a complex of proteins, called the **Mad/Bub** proteins, that bind to **Cdc20** (a component of APC)
- The **Mad/Bub proteins** are assembled in a **complex** at **unattached kinetochores**
- The presence of these proteins (especially Mad2) at an *unattached kinetochore* sends a ***"wait" signal*** to the cell cycle machinery that prevents the cell from continuing on into anaphase.
- The **Mad proteins** are activated in this complex, and then released in an active form that inhibits **Cdc20**, maintaining the **APC in an inactive state**.
- Once all chromosomes are aligned on the spindle i,e microtubules have attached to the kinetochores, the **Mad/Bub complex disassembles** and inhibition of Cdc20 is relieved, leading to **APC** activation.
- **APC** ubiquitinates cyclin B, leading to its degradation and inactivation of Cdkl.
- In addition, **APC** ubiquitinates **securin**, leading to activation of **separase**.
- **Separase degrades** a **subunit of cohesin**, breaking the link between sister chromatids and initiating anaphase

Unattached kinetochores lead to the assembly of a complex of Mad/Bub proteins in which Mad proteins are activated and prevent APC activation by inhibiting Cdc20





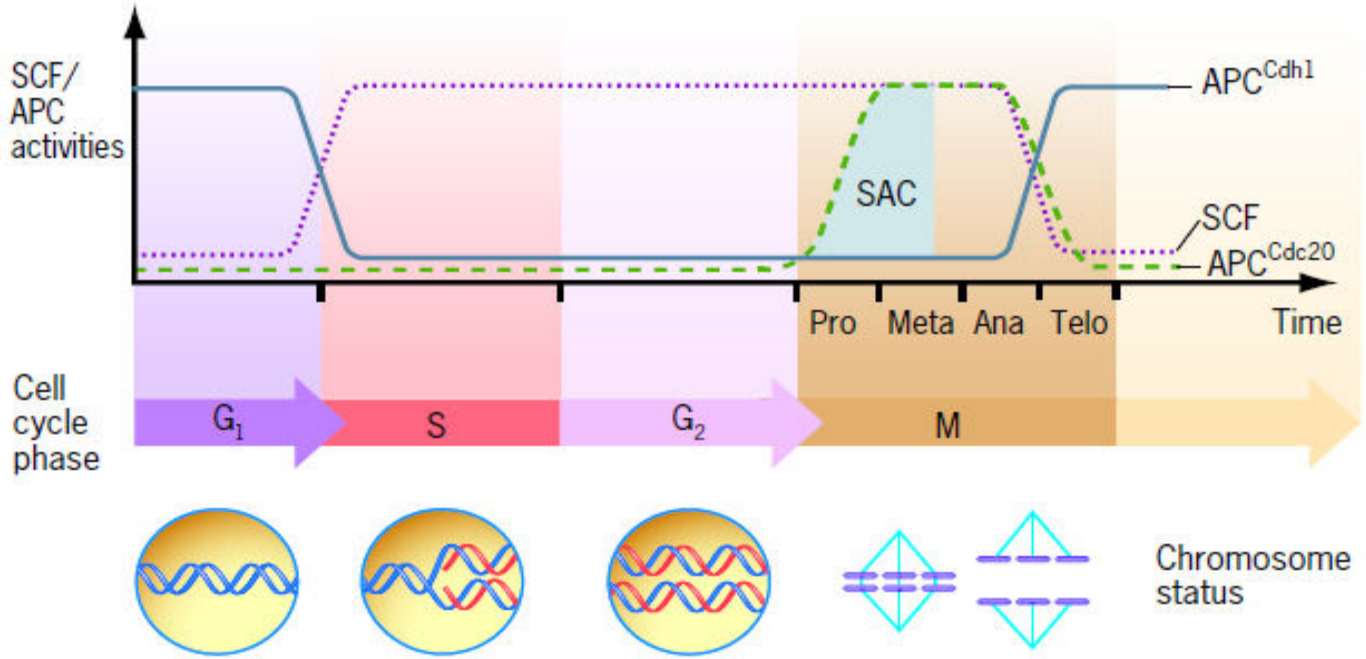
The spindle assembly checkpoint

SCF & APC

- Two distinct multiprotein complexes, SCF and APC, add ubiquitin to proteins at different stages of the cell cycle, targeting them for destruction by a proteasome.

- SCF acts primarily during interphase

- APC is active during mitosis & G1



Two different versions of APC are indicated. These two APCs differ in containing either a Cdc20 or a Cdh1 adaptor protein, which alters the substrates recognized by the APC.

APC^{Cdc20} is active early in mitosis, at a time when **Cdh1 is inhibited** by **Cdk1-mediated phosphorylation.**

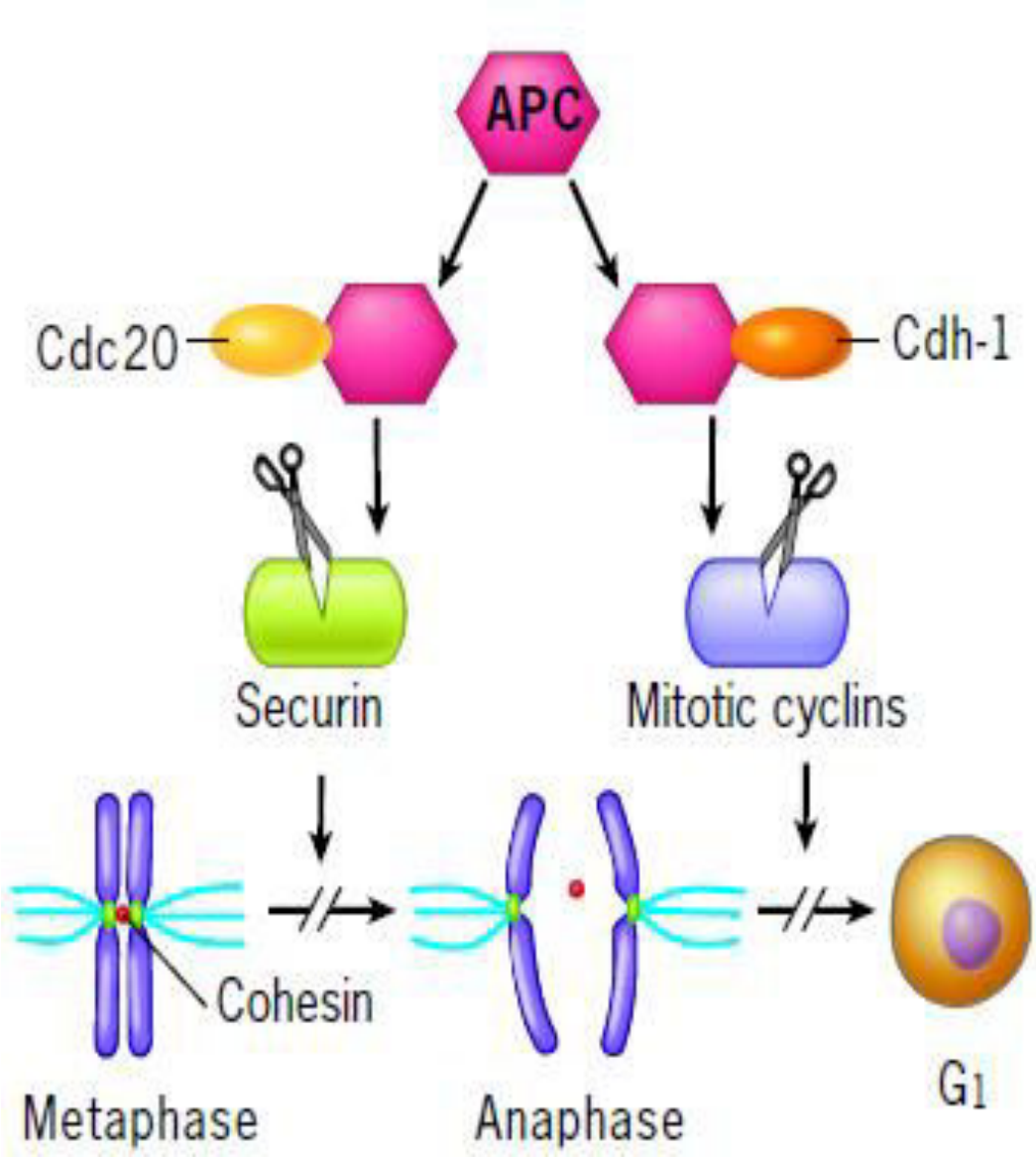
As **Cdk1 activity** drops sharply in late mitosis**, Cdh1** is **activated,** leading to the **activation of APC^{Cdh1}**

The **SAC** prevents **APC^{Cdc20}** from triggering **anaphase** until all the chromosomes are properly aligned at the metaphase plate.

- The APC contains about a dozen core subunits, in addition to an “adaptor protein” that plays a key role in determining which proteins serve as the APC substrate.
- Two alternate versions of this adaptor protein — **Cdc20** and **Cdh1** — determine substrate selection during mitosis
- APC complexes containing one or the other of these adaptors are known as **APC^{Cdc20}** or **APC^{Cdh1}**

Role of Securin & Separare

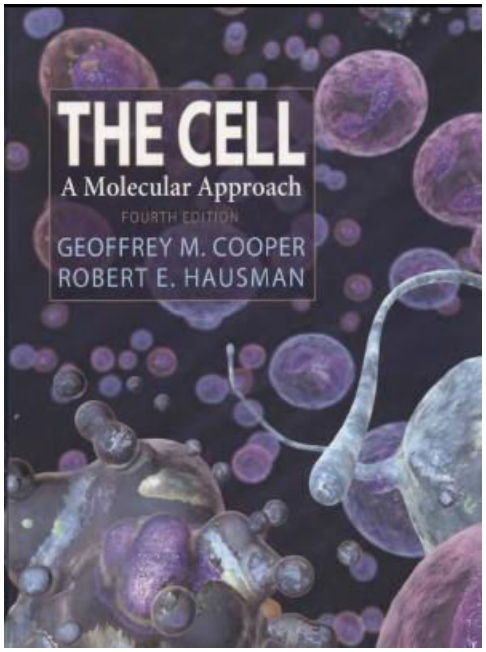
- **APC^{Cdc20}** becomes activated prior to metaphase and ubiquitinates a key anaphase inhibitor called **securin**—so named because it secures the attachment between sister chromatids.
- The ubiquitination and destruction of securin at the end of metaphase release an active protease called **separase**.
- Separase then cleaves the **Scc1** subunit of the **cohesin** molecule that holds sister chromatids together
- Cleavage of cohesin triggers the separation of sister chromatids to mark the onset of anaphase (movement towards the poles).



APC^{Cdc20} is responsible for destroying proteins, such as securin, that inhibit anaphase. Destruction of these substrates promotes the metaphase–anaphase transition

- Near the end of mitosis, Cdc20 is inactivated, and the alternate adaptor, Cdh1, takes control of the APC's substrate selection
- When Cdh1 is associated with the APC, the enzyme completes the ubiquitination of cyclin B that was begun by **APC^{Cdc20}**.
- Destruction of the cyclin leads to a precipitous drop in activity of the mitotic Cdk (cyclin B–Cdk1) and progression of the cell out of mitosis and into the G1 phase of the next cell cycle.
- If the destruction of cyclin B is prevented with an inhibitor of the proteasome, cells remain arrested in a late stage of mitosis.

Reference: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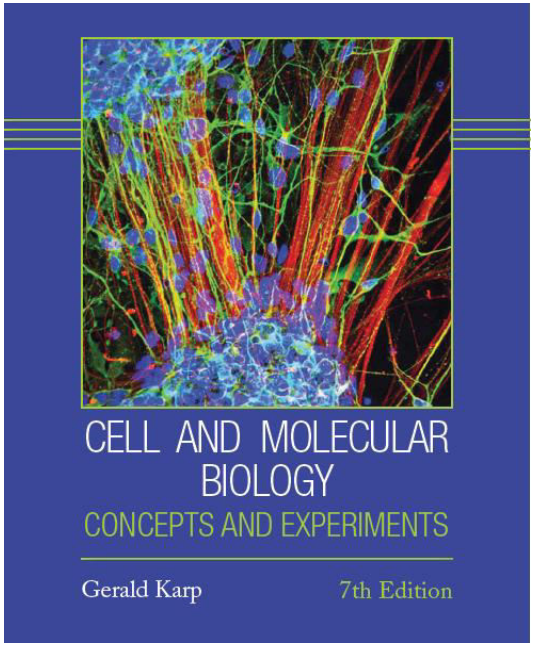
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