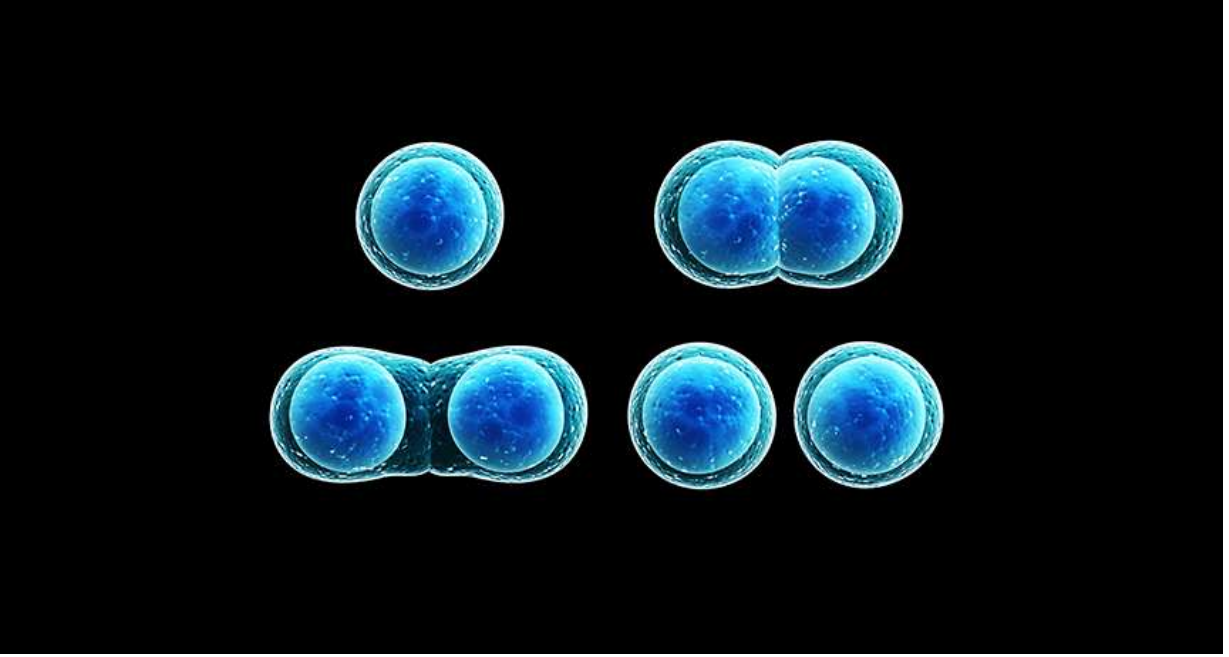
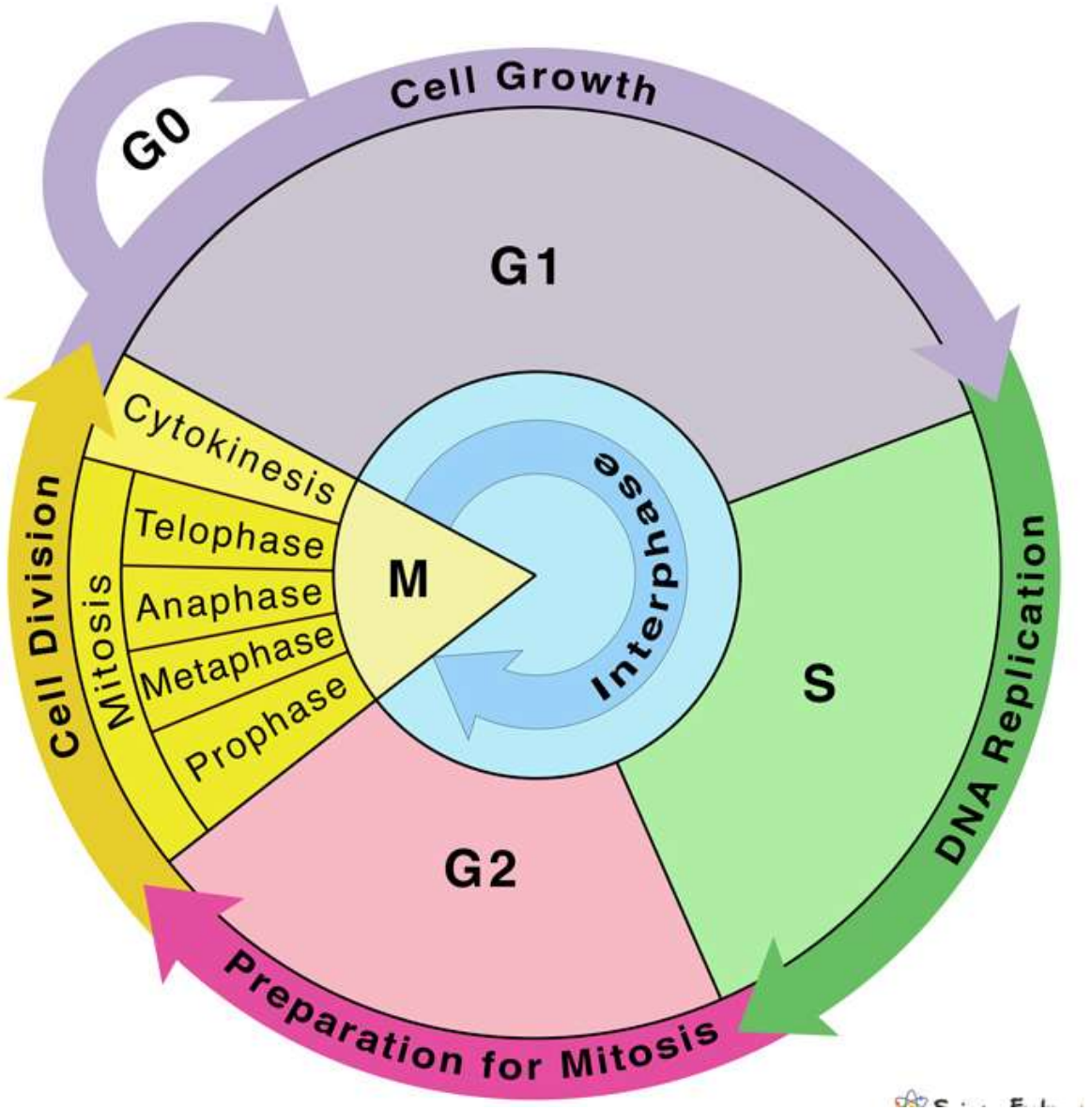


Cell cycle and its regulation

Cell Cycle



- **M phase**: duplicated chromosomes are separated into two nuclei, and entire cell divides into two daughter cells
- **Interphase**: is a time when the cell grows and engages in diverse metabolic activities
- **G1 phase**: Cell growth
- **S phase**: DNA replication and Chromosome duplication
- **G2 phase**: Cell grows and prepares for mitosis
- **G0:** Quiescent stage

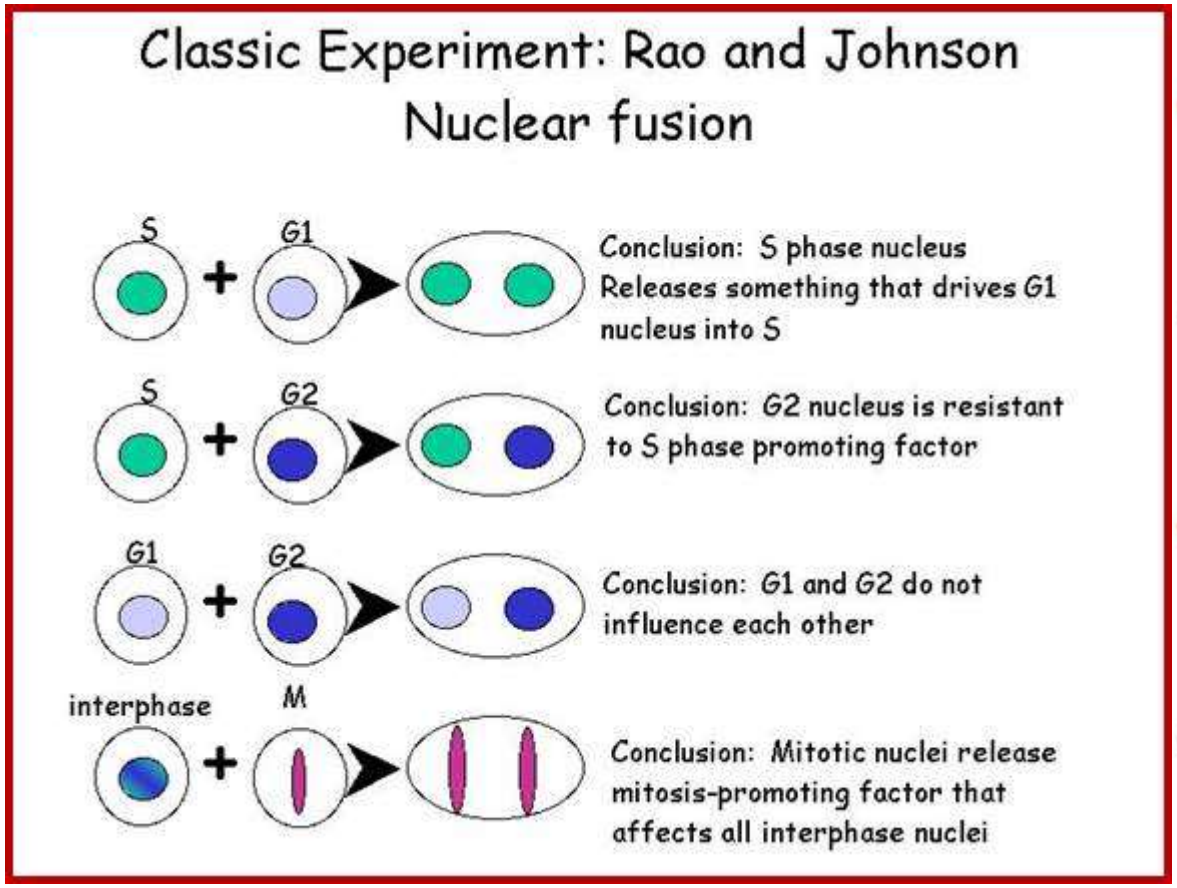
Cell Cycles in Vivo

- One of the properties that distinguishes various types of cells within a multicellular plant or animal is their capacity to grow and divide

- We can recognize three broad categories of cells:

1. Cells, once differentiated, they remain in that state until they die. Eg: nerve cells, muscle cells, or red blood cells, lack the ability to divide
2. Cells that normally do not divide but can be induced to begin DNA synthesis and divide when given an appropriate stimulus. Eg: liver cells, lymphocytes
3. Cells that normally possess a relatively high level of mitotic activity. Eg: Stem cells

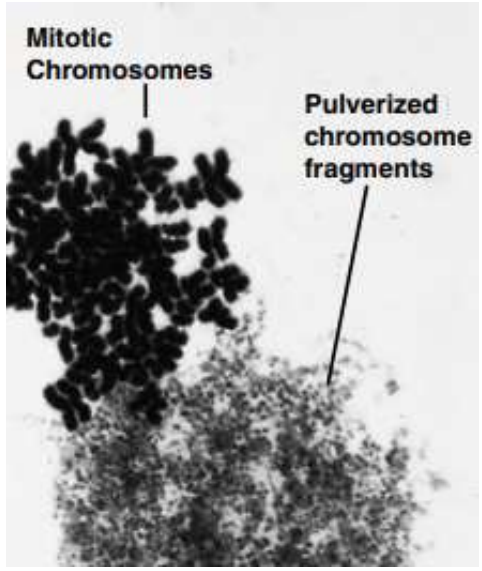
Cell fusion experiment

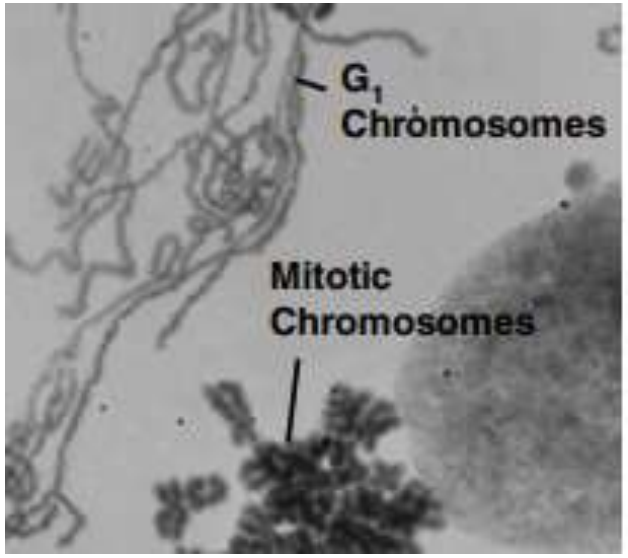


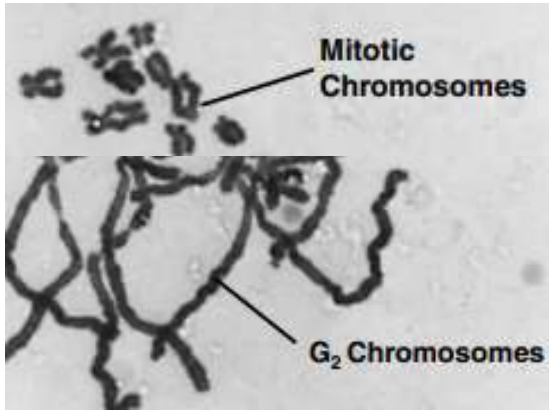
- G1 cells were fused with S phase, G1 abruptly resumed DNA synthesis and entered S phase
- Thus cytoplasm of S phase contained factors that initiated DNA synthesis in S phase
- S phase cells were fused with G2 phase, it was unable to synthesis DNA but its progressed towards M
- G1 fused with G2- no effect but G2 to M was delayed

Cell fusion experiment

- G1→M: the chromatin of the G1-phase showed premature chromosomal compaction
- G2→M: G2 chromosomes: premature chromosome compaction, doubled
- S→M: S-phase: “pulverized” chromosomal fragments







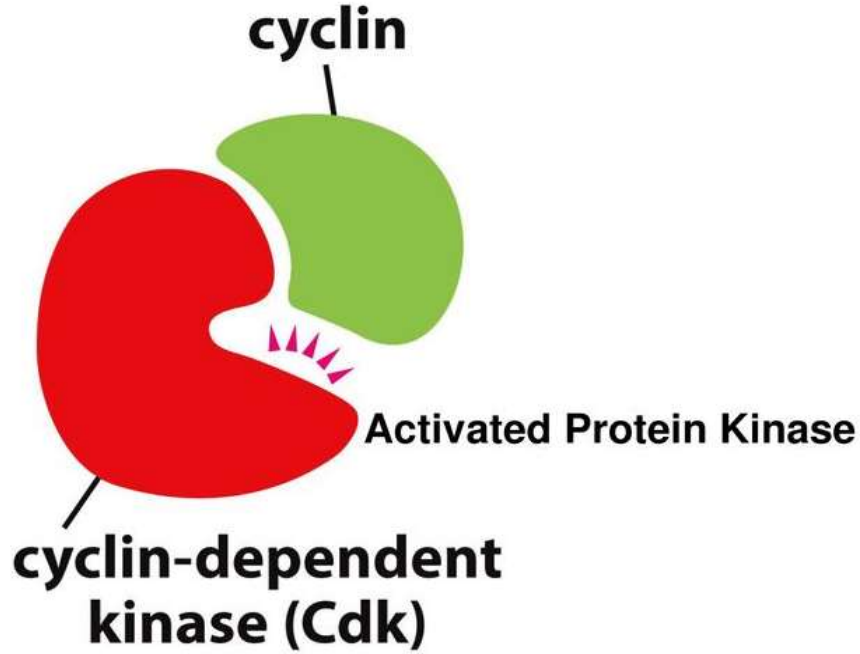
Role of Protein Kinases

- Entry of a cell into M phase is initiated by a protein called maturation promoting factor (MPF)

- MPF consists of two subunits: (1) a subunit with kinase activity that transfers phosphate groups from ATP to specific serine and threonine residues of specific protein substrates→ CDKs (cyclin-dependent kinases)

- The activity of this enzyme is controlled by a subunit whose concentration varies from one stage of the cell cycle to another i.e cyclin
  (2) a regulatory subunit called cyclin

CDK



- Constitute family of Protein kinases
- Add phosphate group to target
- Regulated by cyclin
- Catalyse phosphorylation of serine and threonine residues of specific target proteins
- Reversible by phosphatases
- CDKs possess two tyrosine phosphorylation sites: One causes activation of the enzyme; the other causes inactivation.
- Specific kinases carry out both the stimulatory and the inhibitory phosphorylation

Regulation of CDKs

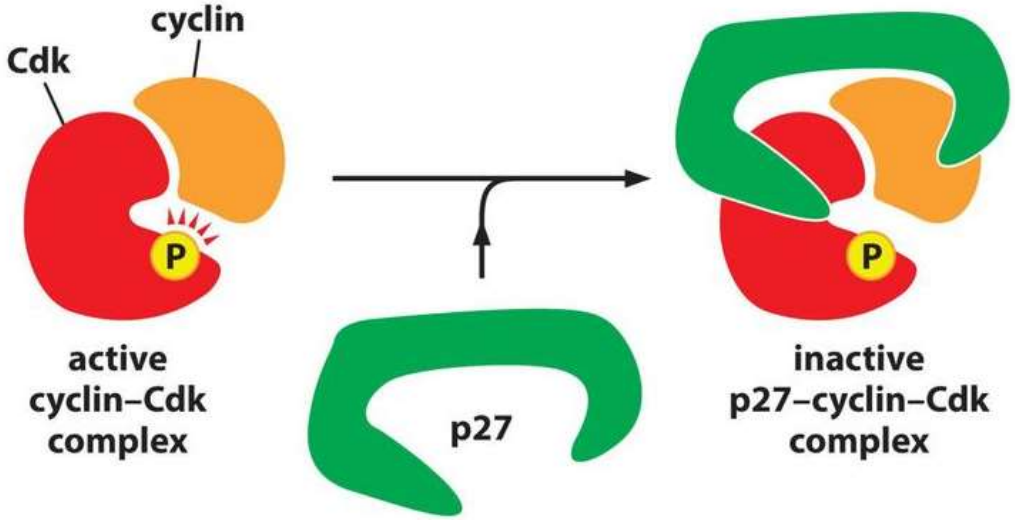
- The activities of CDKs are regulated by a variety of “brakes” and “accelerators” that operate in combination with one another. These include:

➢ **Cyclin Binding**

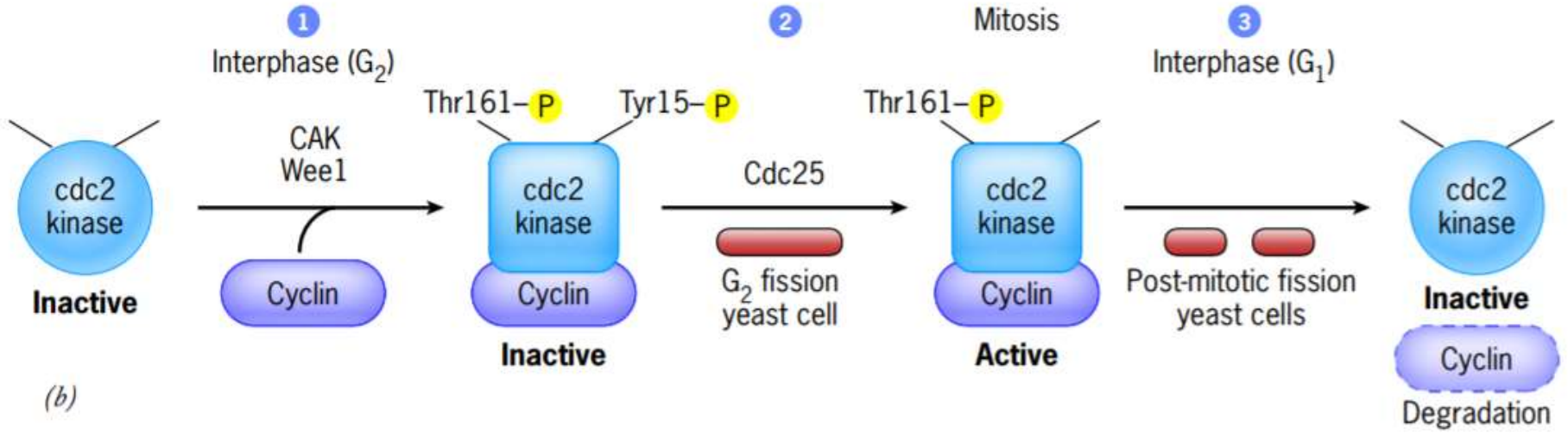
➢ **Cdk Phosphorylation/dephosphorylation**

➢ **Cdk Inhibitors**

- Cdk activity can be blocked by a variety of inhibitors
- In budding yeast, for example, a protein called Sic1 acts as a Cdk inhibitor during G1
- In mammalian cells, Cdk inhibitors examples are p21 and p27



Cdk Phosphorylation/dephosphorylation



- **Step 1**: CAK (Cdk-activating kinase), phosphorylates a critical threonine residue (**Thr 161 of cdc2/cdk1**)
- A second protein kinase, called Wee1, phosphorylates a key tyrosine residue in the ATP-binding pocket of the enzyme (**Tyr 15 of cdc2/cdk1**)
- **Step 2**: Phosphate at Tyr 15 is removed by a phosphatase named Cdc25
- **Step 3**: Removal of this phosphate switches the stored cyclin–Cdk molecules into the active state, allowing it to phosphorylate key substrates and drive the cell into mitosis.

Regulation of CDKs

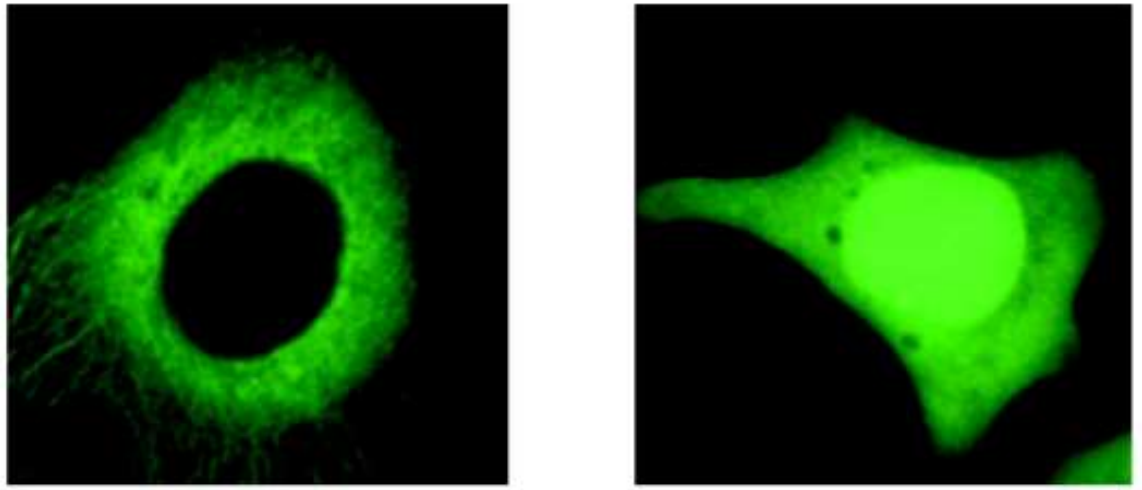
➢ **Controlled Proteolysis**:

- Degradation is accomplished by means of the ubiquitin–proteasome pathway by ubiquitin ligases (SCF and APC complexes)
- APC complex acts in mitosis (M cyclin) and SCF acts during G1 phase (G1 cyclin)

Regulation of CDKs

➢ **Subcellular Localization**:

- Subcellular localization is a dynamic phenomenon in which cell cycle regulators are moved into different compartments at different stages
- For example, one of the major mitotic cyclins in animal cells (cyclin B1) shuttles between the nucleus and cytoplasm until G2, when it accumulates in the nucleus just prior to the onset of mitosis

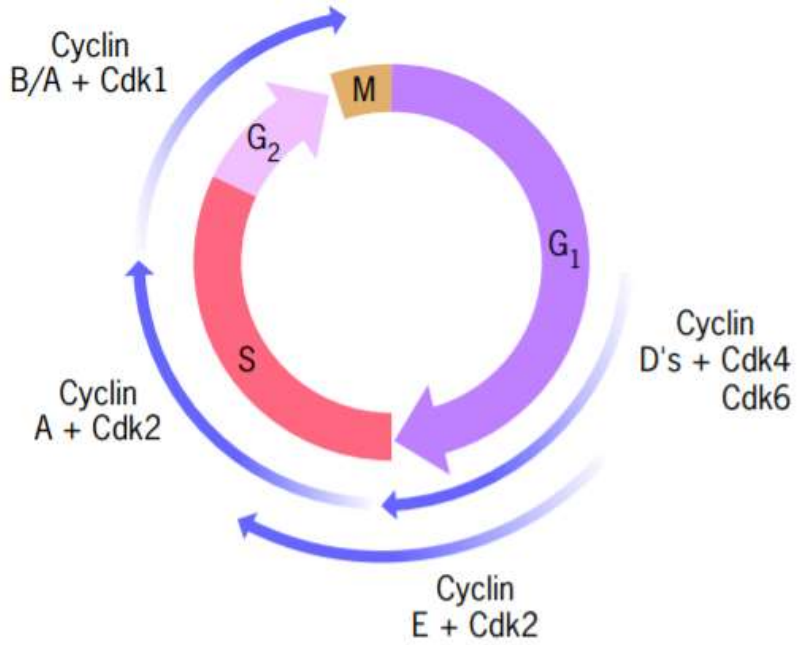


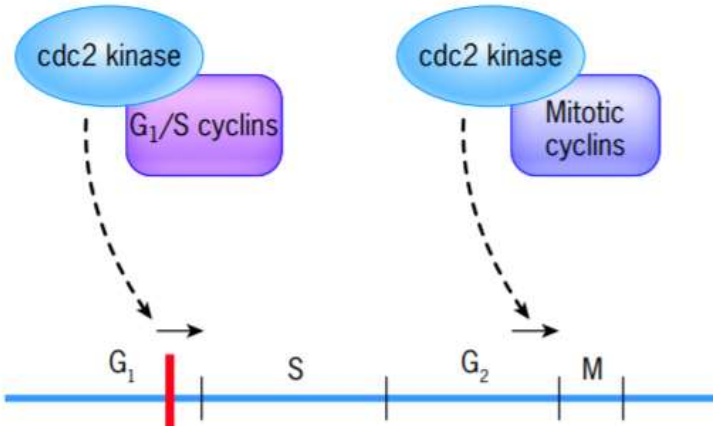
(a) (b)

Figure 14.7 **Experimental demonstration of subcellular localization during the cell cycle.** Micrographs of a living HeLa cell that has been injected with cyclin B1 linked to the green fluorescent protein (page 273). The cell shown in *a* is in the G_2 phase of its cell cycle, and the fluorescently labeled cyclin B1 is localized almost entirely in the cytoplasm. The micrograph in *b* shows the cell in prophase of mitosis, and the labeled cyclin B1 is concentrated in the cell nucleus. The basis for

Cyclin-CDKs in the mammalian cell cycle

CDKs	Cyclin	Stage
Cdk4, Cdk6	Cyclin D's (D1, D2, D3)	Mid G1 phase
Cdk2	Cyclin E	Transition from G1 to S phase (late G1)
Cdk2	Cyclin A	S phase
Cdk1 (cdc2- fission yeast)	Cyclin B	M phase





Cell cycle regulation in fission yeast

DNA Damage Checkpoints

•DNA damage checkpoints ensure the fidelity of genetic information both by arresting cell cycle progression and facilitating DNA repair pathways.

•Studies on many different species have uncovered a network of proteins that form the DNA damage checkpoints.

•Central to this network are protein kinases of ATM/ATR family known as Tel1/Mec1 in budding yeast and Tel1/Rad3 in fission yeast.

•These kinases sense DNA damages and phosphorylate number of proteins that regulate cell cycle progression and DNA repair pathways

DNA Damage Checkpoints

- ATM (Ataxia Telangiectasia Mutated) and ATR (Ataxia Telangiectasia and Rad3 related) are closely related kinases that are activated by DNA damage.

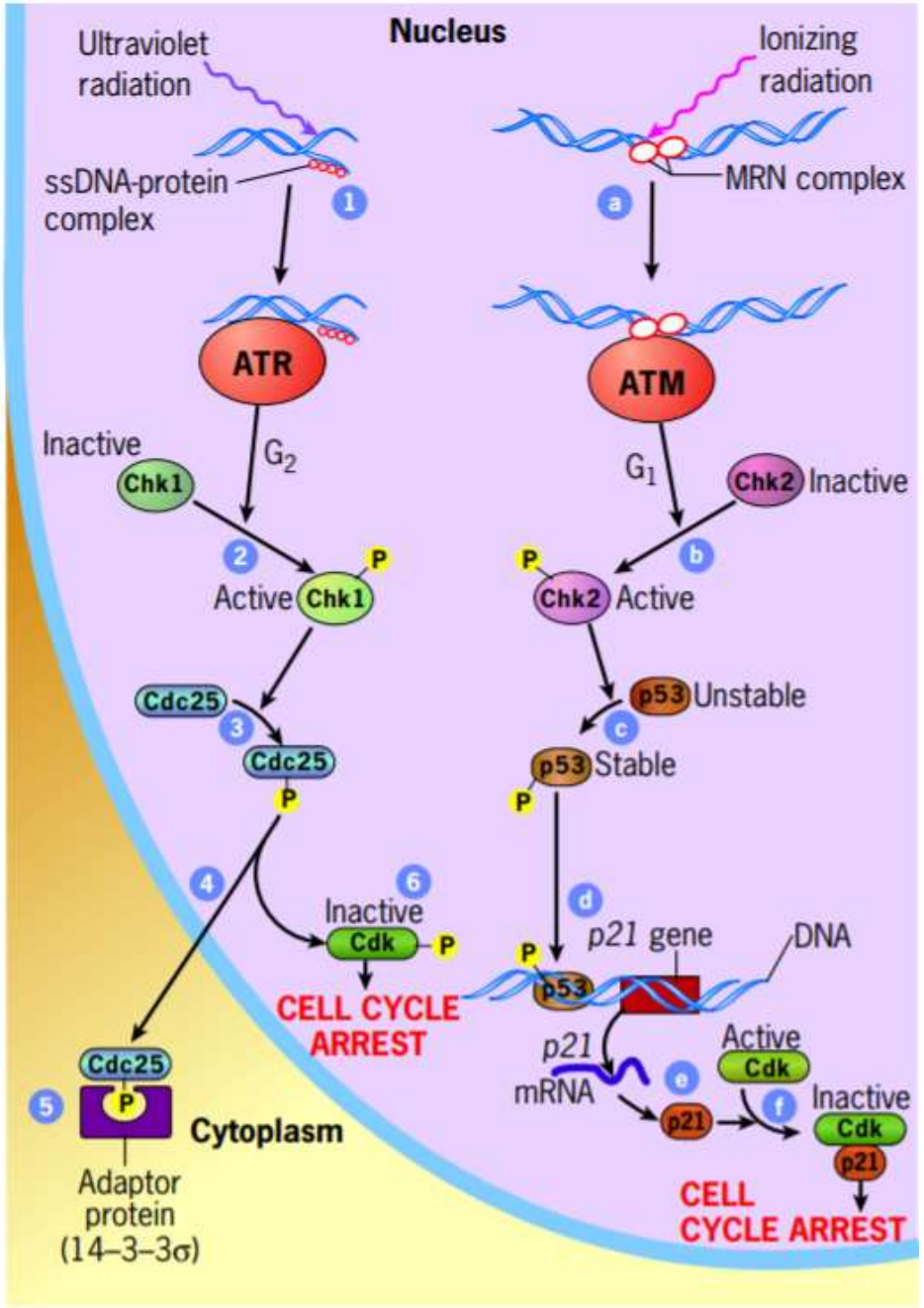
- These serine-threonine protein kinases are members of phosphatidyl inositol 3' kinase like kinase family (PIKK)

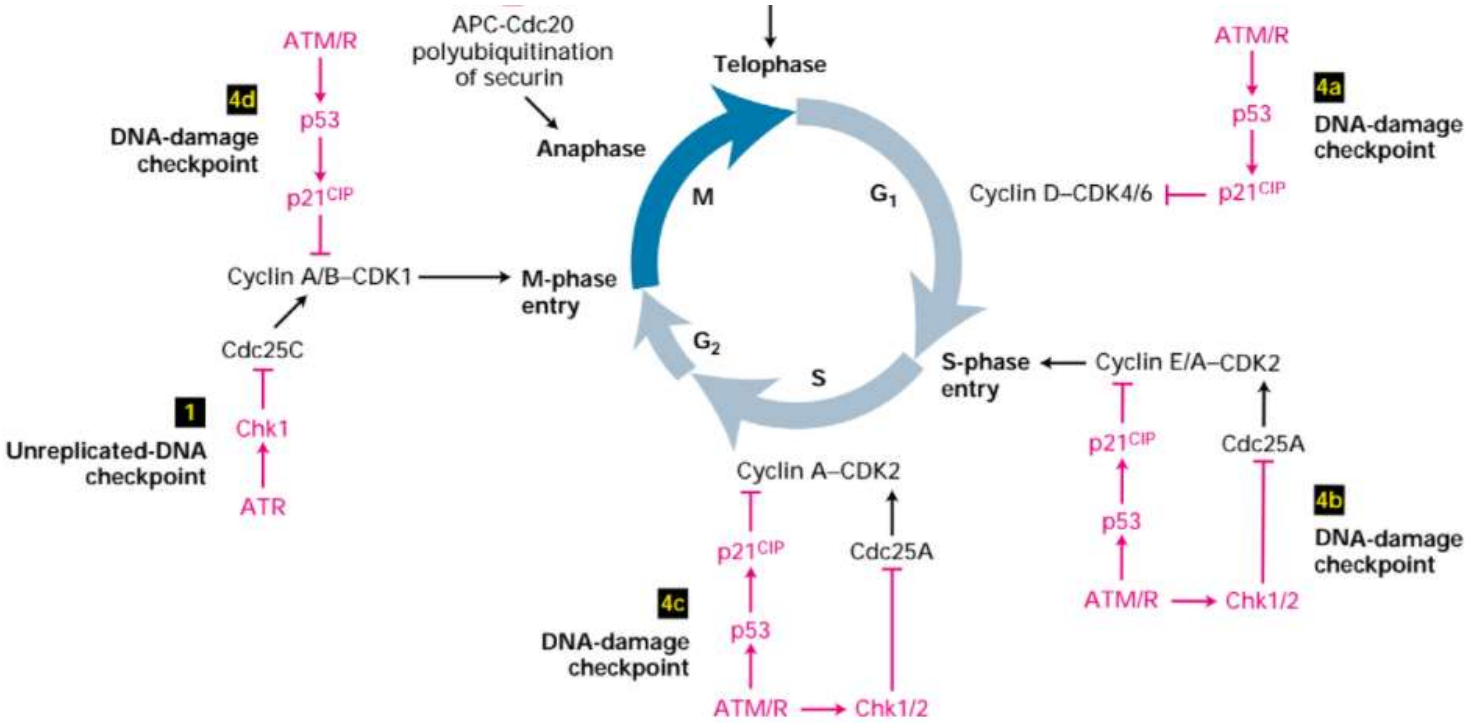
- Whereas ATM is primarily activated by double-stranded DNA breaks (DSBs), ATR responds to a broad spectrum of DNA damage, including DSBs and a variety of DNA lesions that interfere with replication.

- In mammals, MRN (Mre11/Rad50/Nbs1) complex has been proposed as ds break sensor in ATM pathway

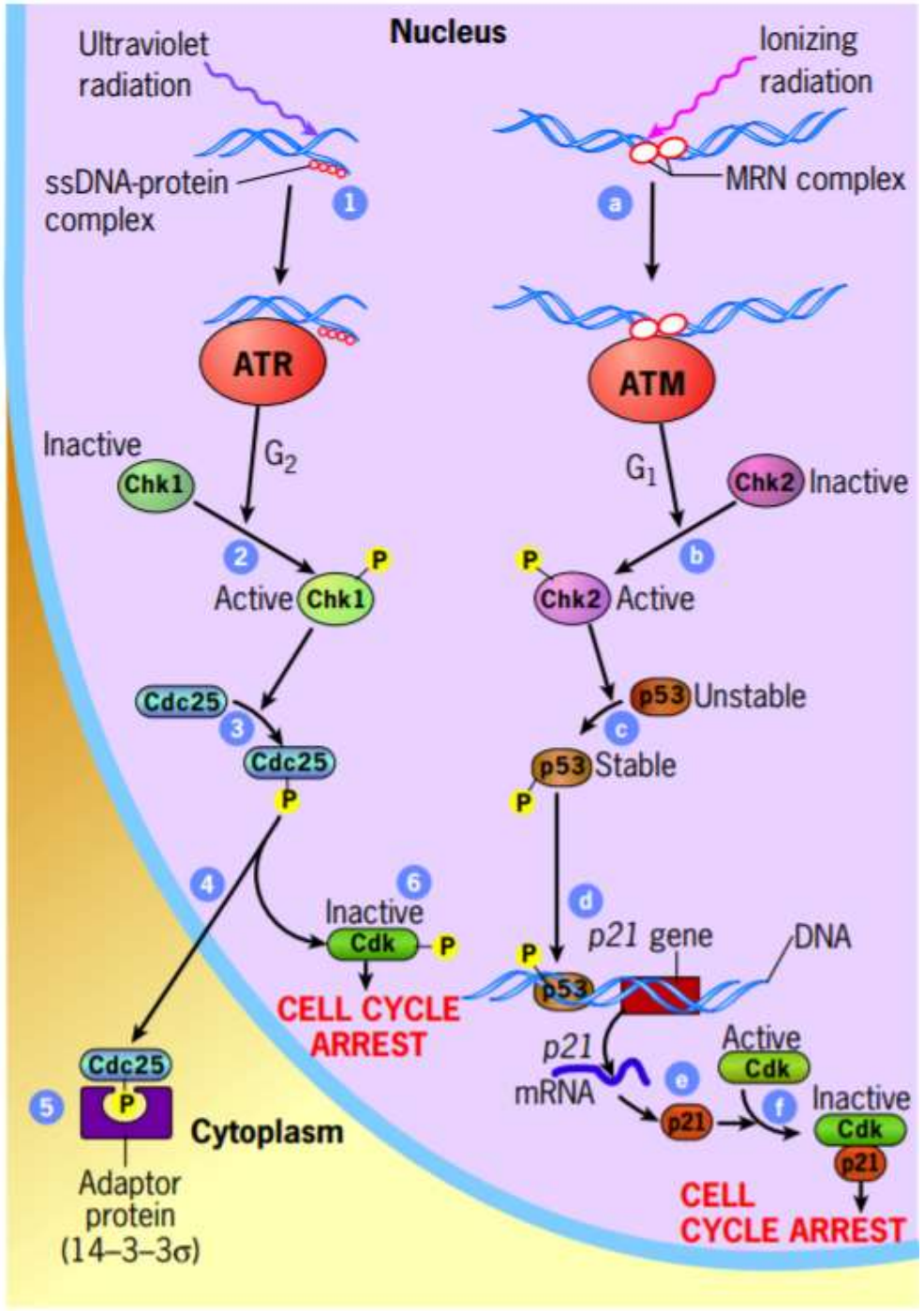
- ATM binds MRN and activates ATM kinases – phosphorylation of kinase chK2 (checkpoint kinases 2) activate p53 and leads to cell cycle arrest/ DNA repair/ Apoptosis

DNA Damage Checkpoints

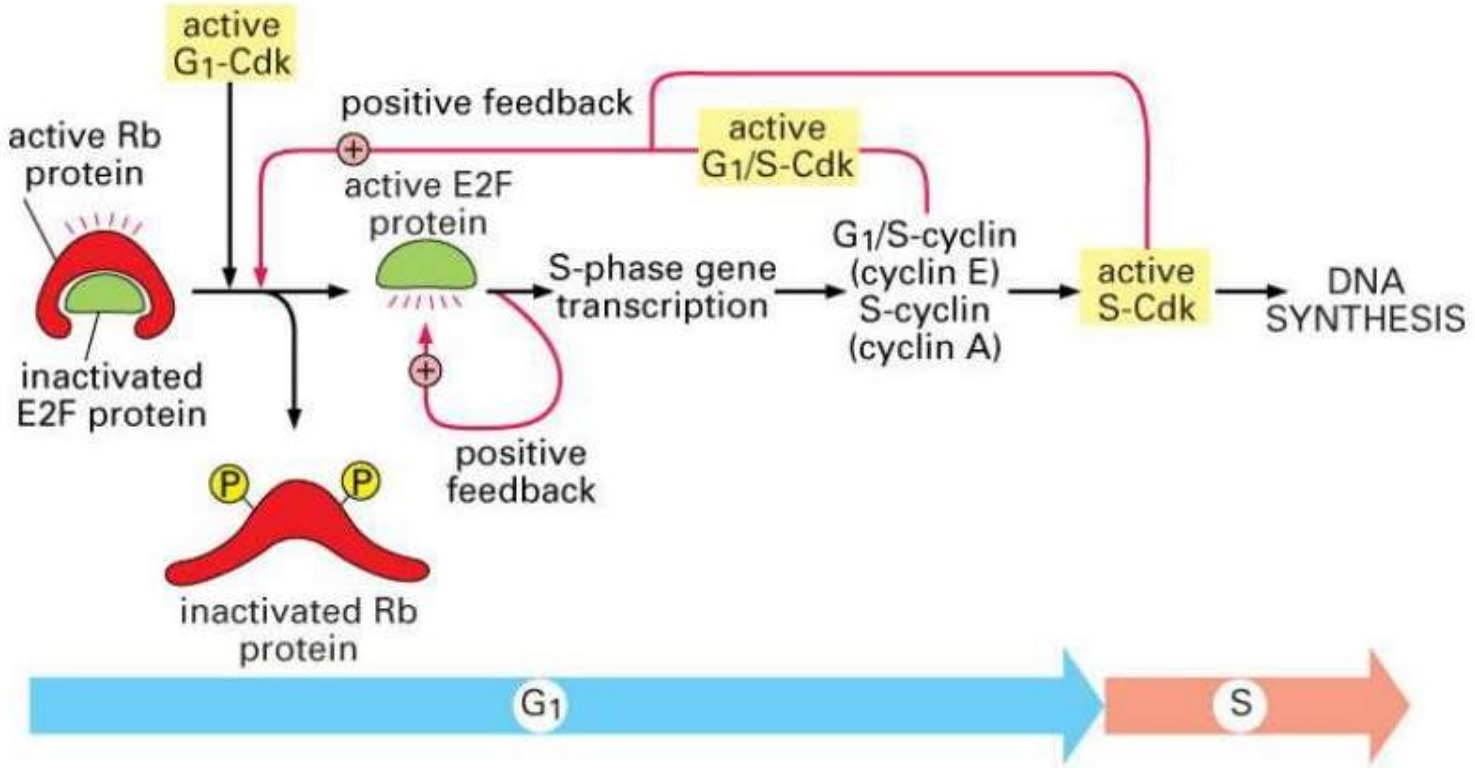




DNA Damage Checkpoints



Control Of G_1 Progression By Retinoblastoma Protein

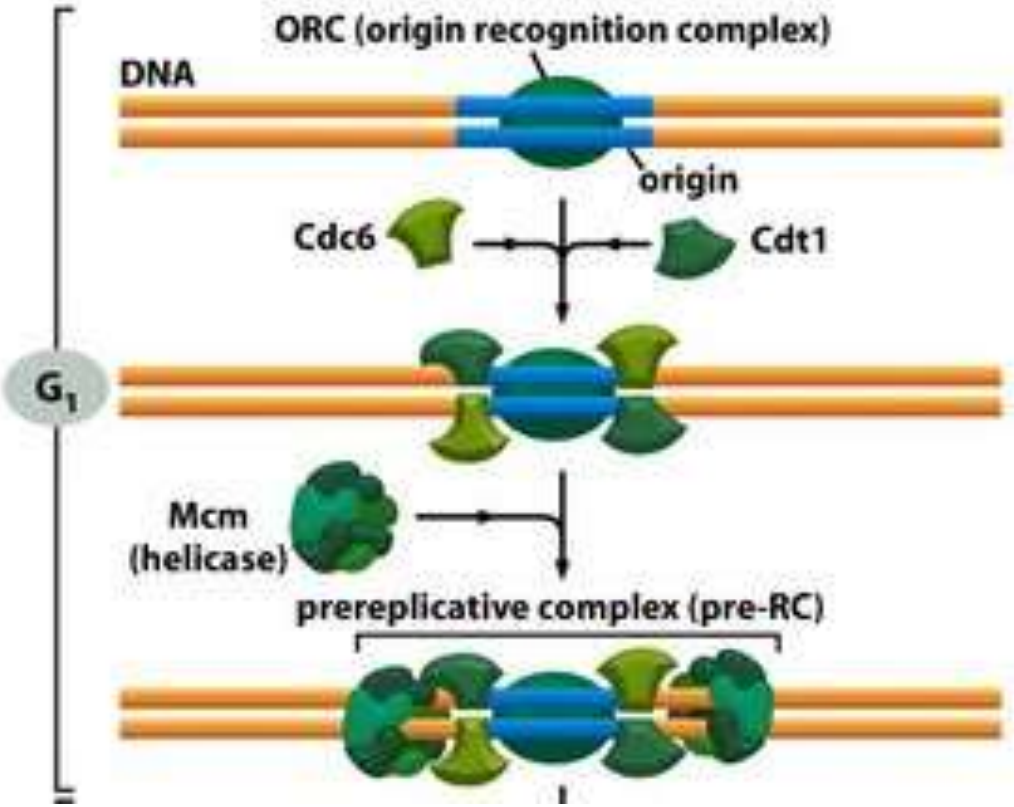


- G_1-cyclin-Cdk phosphorylates Rb, resulting in release of E2F and transcription of S phase genes

- E2F transcription factors stimulate transcription of genes encoding the late-G1 cyclin (cyclin E), the S-phase cyclin (cyclin A), and the S-phase CDK (CDK2).

Control of DNA Replication in S-phase

- Origin recognition complex (**ORC**) is a multiprotein complex that binds to replication origin
- ORC bind to replication origins throughout the cell cycle and serve as landing pads for several additional regulatory proteins
- In **early G1**, the proteins **cdc6** and **cdt1** bind to the ORC at origins
- This leads to the binding of the **Mcm** proteins complex (helicase) which is composed of a group of 6 closely related proteins (Mcm 2-7)
- The resulting large complex is the **pre-RC**, and the origin is now licensed for replication.

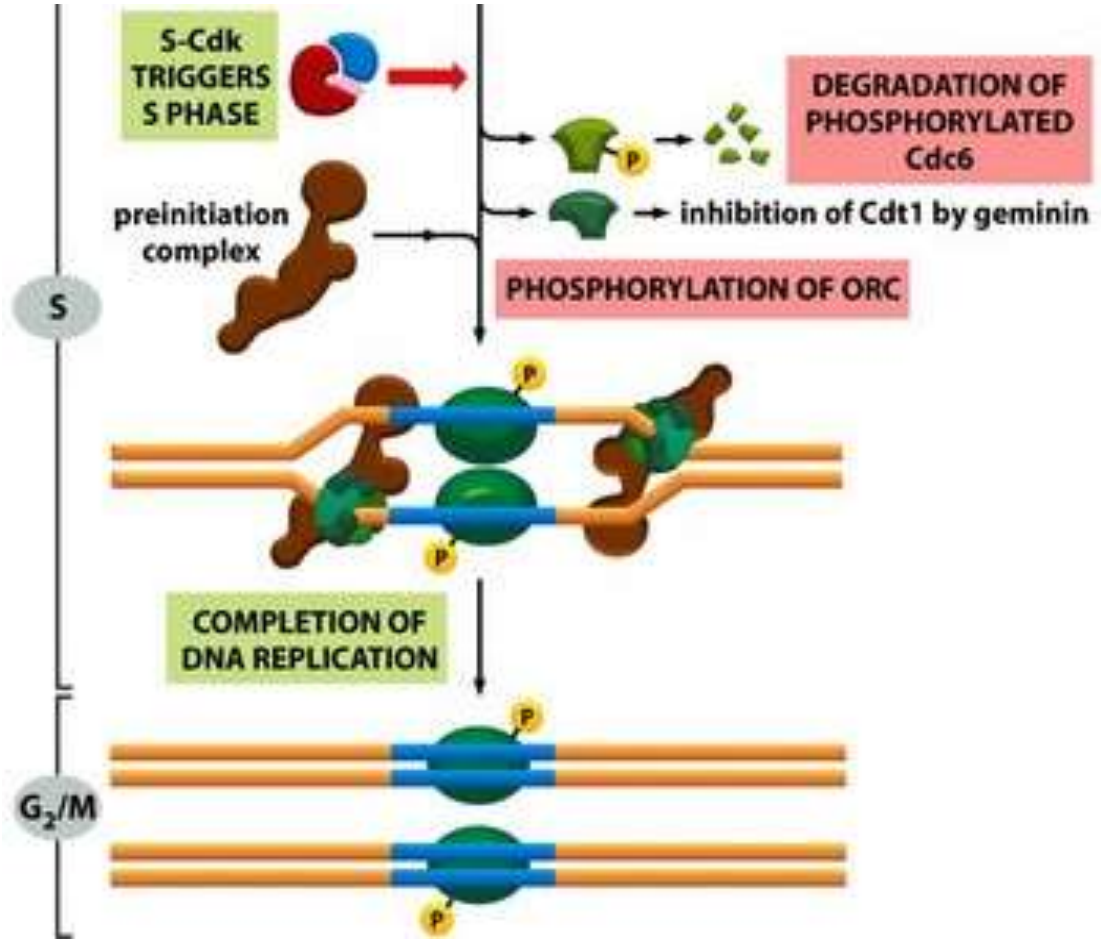


Control of DNA Replication in S-phase

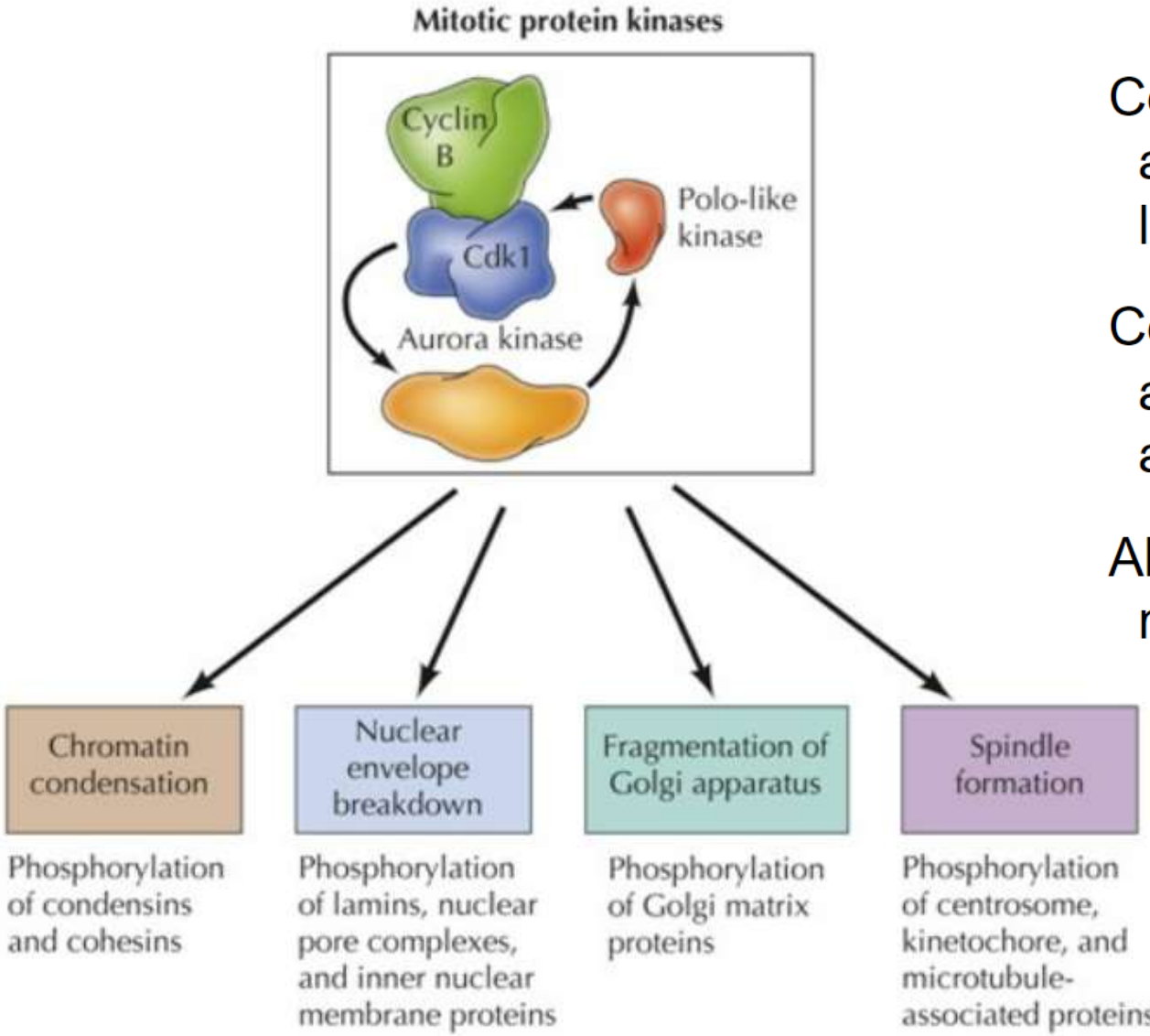
- The activation of S-Cdk in late G1 initiates DNA replication:
  - S-cdk phosphorylates Cdc6 which is then degraded
  - S-cdk trigger the assembly preinitiation complex
  - S-cdk with help of additional kinase collaborate to phosphorylate ORC. DNA synthesis begins

- S-Cdk complex restrain pre-RC assembly and prevent DNA re-replication after S phase:
  - It phosphorylates Cdc6, thereby causing the Cdc6 protein to dissociate from ORC, triggering its ubiquitylation by the SCF enzyme complex and thus its degradation

  - It also phosphorylates excess Mcm proteins, Mcm protein complex cannot bind to a replication origin and this triggers their export from the nucleus.



G2 checkpoint: G2→M transition: cyclin B - cdk1

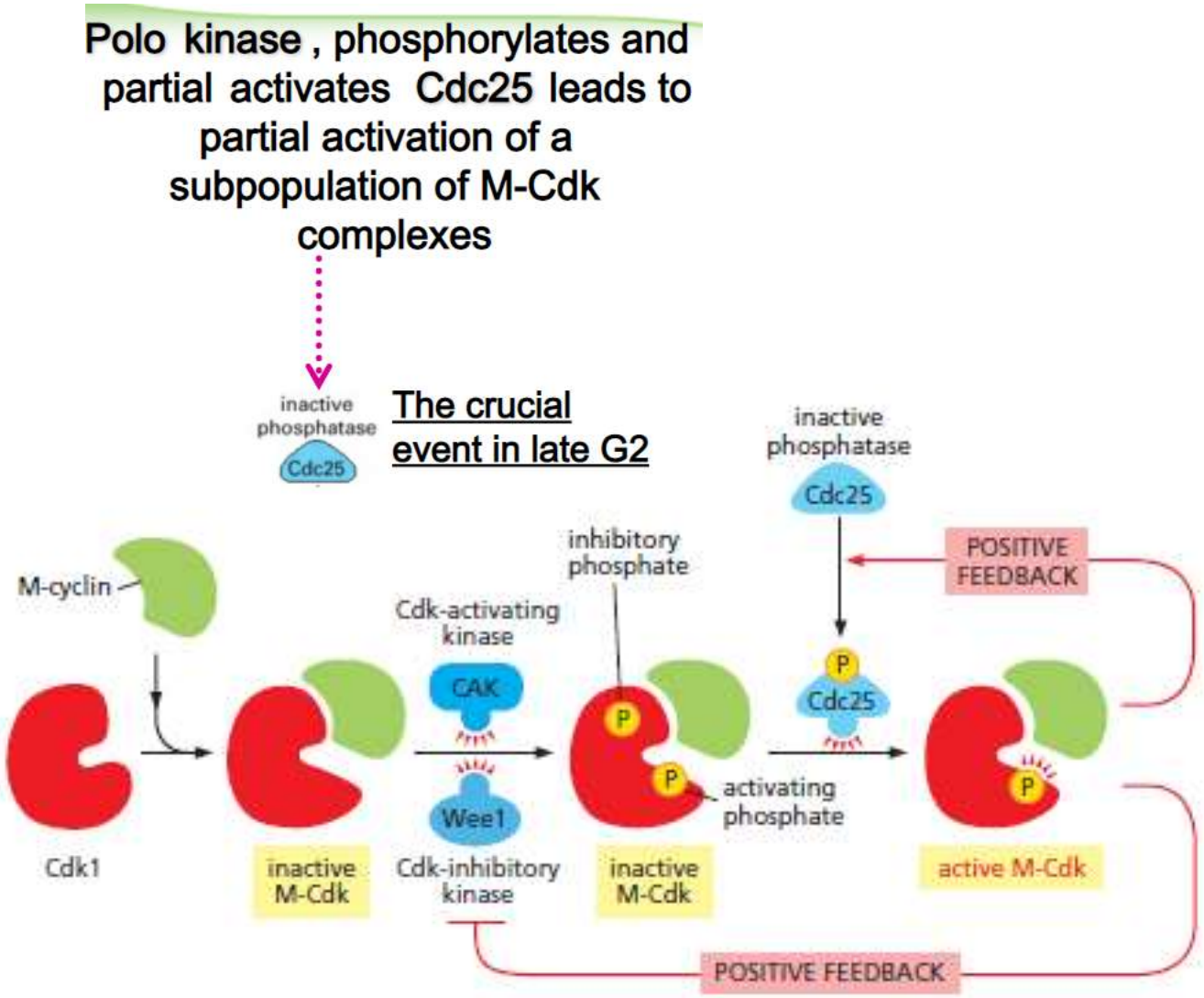


Cdk1, **Aurora** and **Polo-like kinases** are activated in a positive feedback loop to signal entry into M phase.

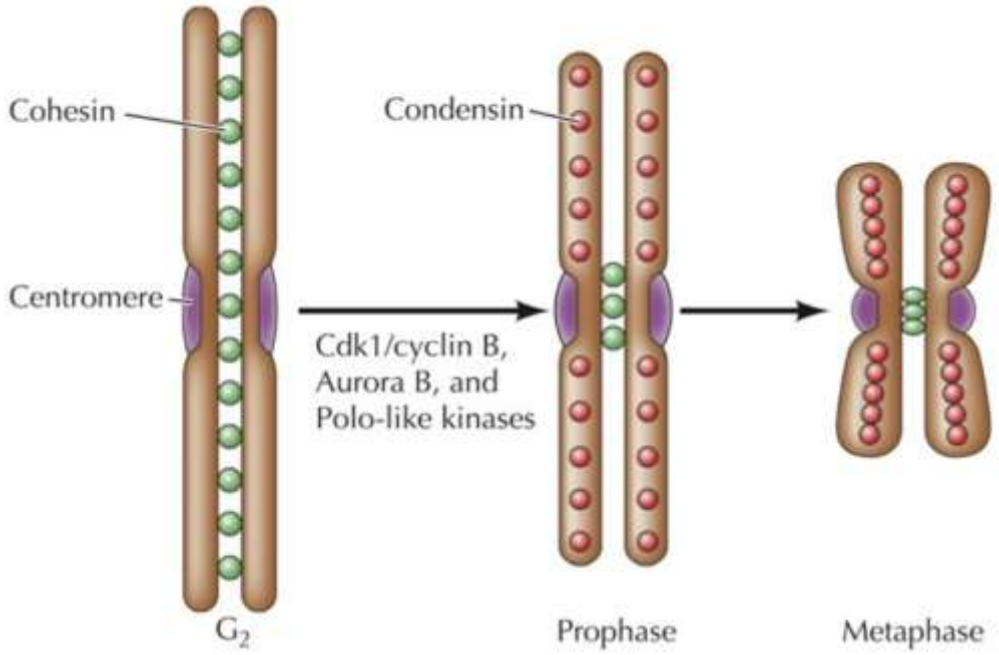
Cdk1 activates Aurora kinases, which activate Polo-like kinases, which in turn activate Cdk1.

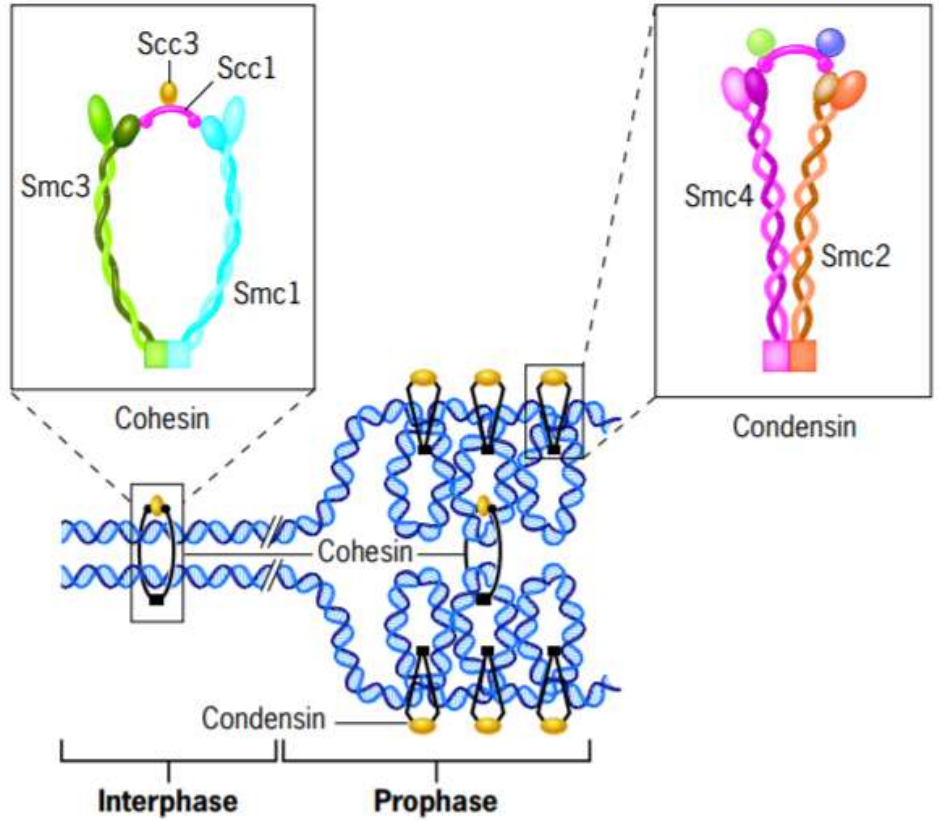
All of these protein kinases have multiple roles in mitosis.

Control of M-Cdk complex until late G2 phase



Chromatin Condensation



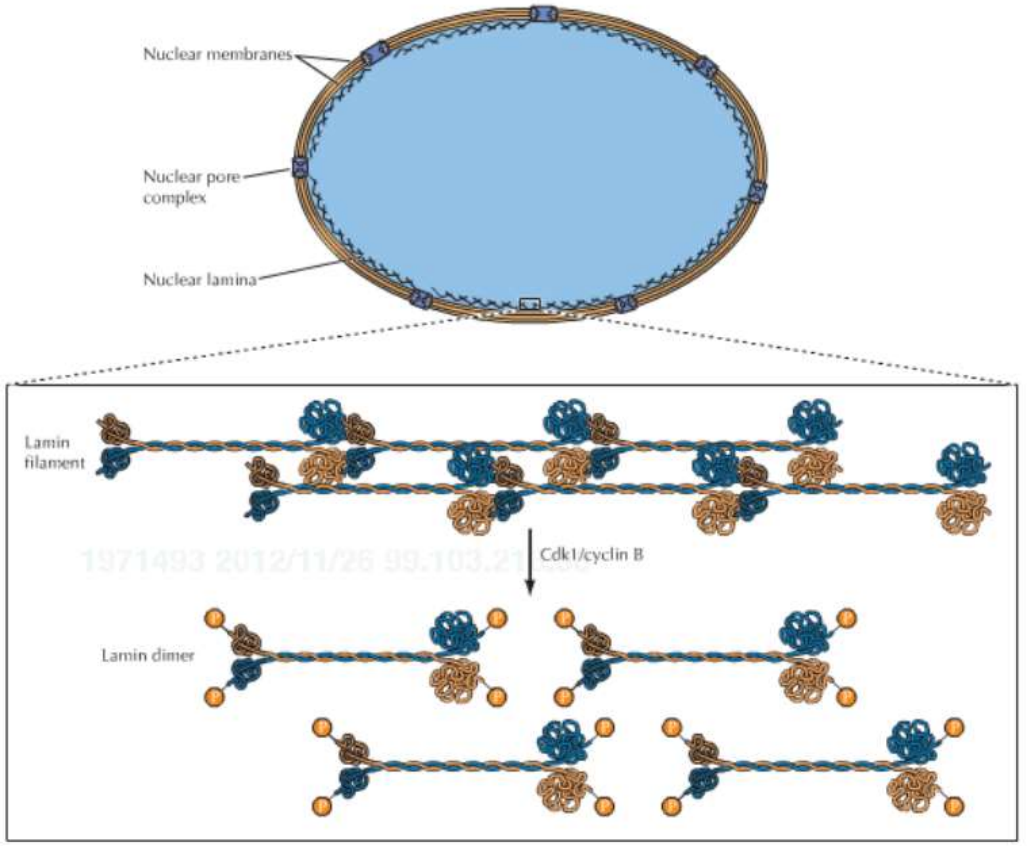


- Condensin is activated at the onset of mitosis by phosphorylation of several of its subunits by the cyclin B–Cdk1
- Dissociation is induced by phosphorylation of cohesin subunits by two mitotic enzymes called Polo-like kinase and Aurora B kinase
- Cohesin remains at the centromeres, and removed during anaphase

Nuclear envelope breakdown

Breakdown of the nuclear envelope involves changes in all components:

- Nuclear membranes fragment
- Nuclear pore complexes dissociate
- Nuclear lamina depolymerizes—due to phosphorylation of lamins by Cdk1/cyclin B

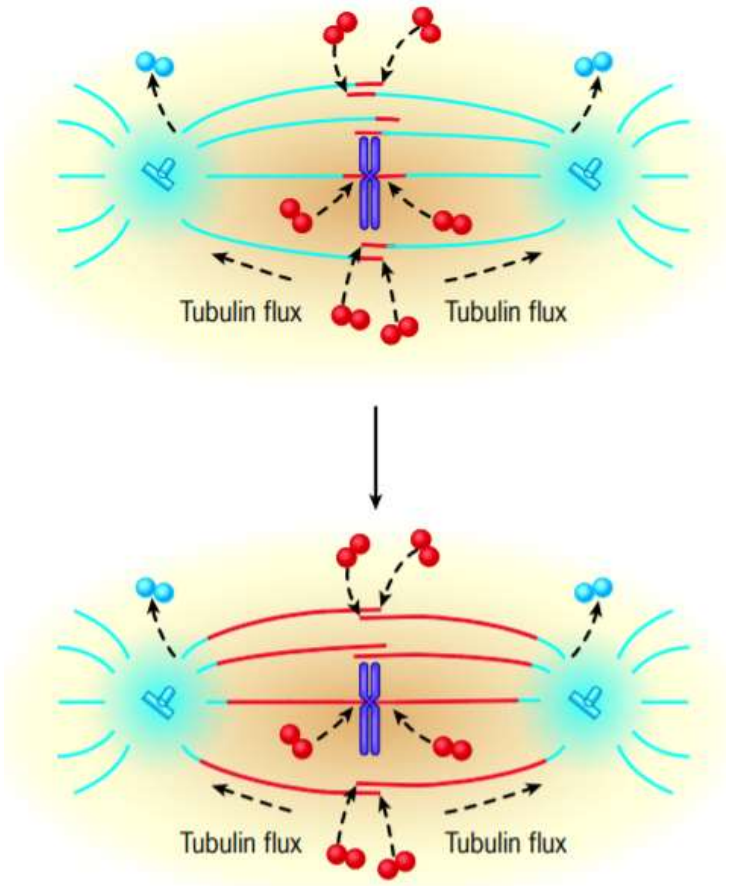


Fragmentation of Golgi apparatus

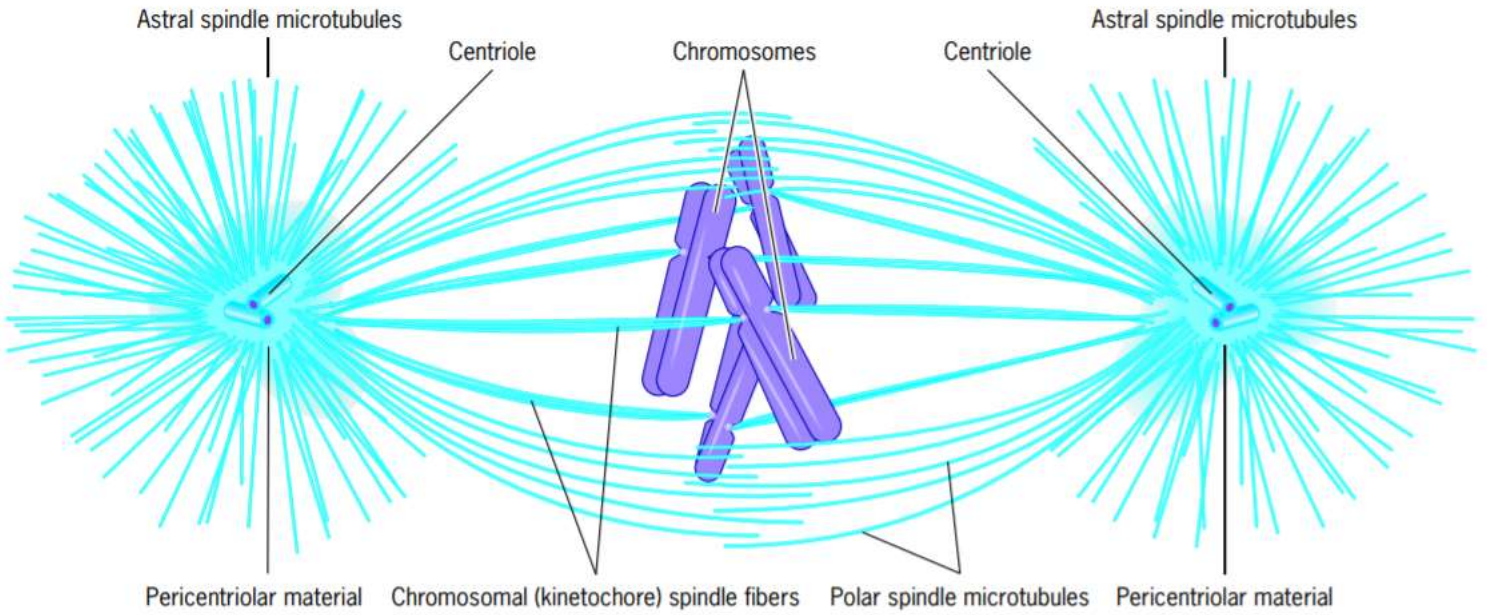
The Golgi apparatus fragments into vesicles, which are absorbed into the ER or distributed to daughter cells at cytokinesis.

Golgi breakdown is mediated by phosphorylation of proteins by Cdk1 and Polo-like kinases.

Formation of the Mitotic Spindle



Centrosome maturation and spindle assembly are driven by Aurora and Polo-like kinases at the centrosomes.



- **Astral** microtubules that radiate outward from the centrosome into the region outside the body of the spindle
- **Chromosomal** (or kinetochore) microtubules that extend between the centrosome and the kinetochores of the chromosomes.
- **Polar** (or interpolar) microtubules that extend from the centrosome past the chromosomes

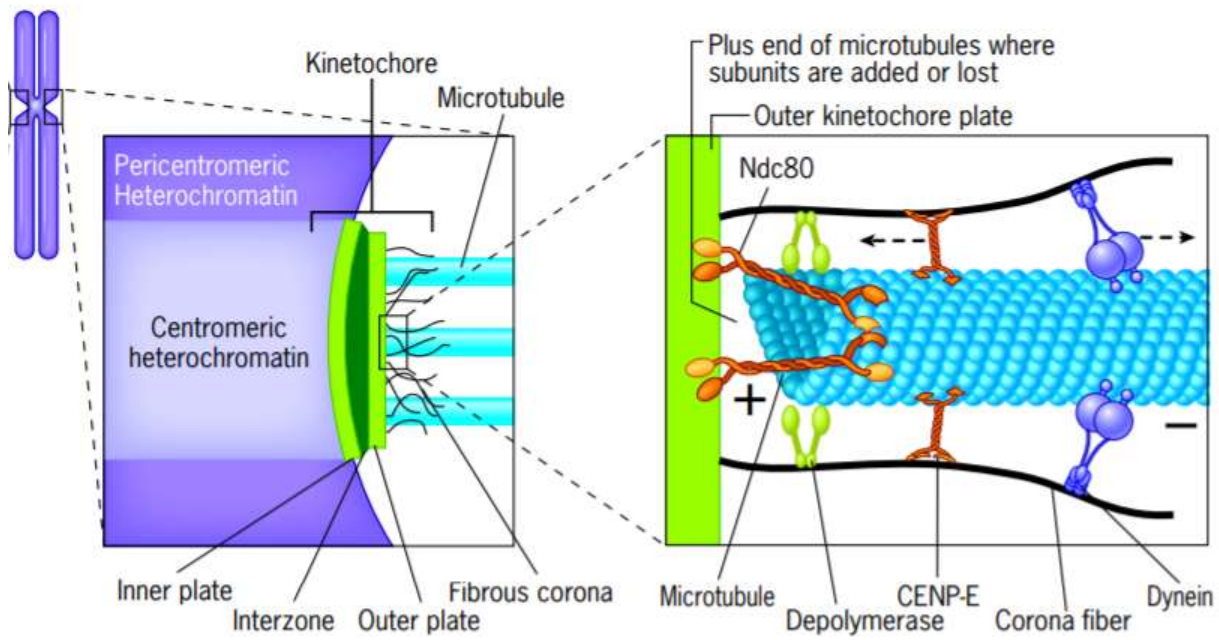
Metaphase

- The centromere is the residence of highly repeated DNA sequences that serve as the binding sites for specific proteins
- Kinetochore is the complex of proteins, at the outer surface of the centromere of each chromatid
- Kinetochore functions as:

(1) the site of attachment of the chromosome to the dynamic microtubules of the mitotic spindle

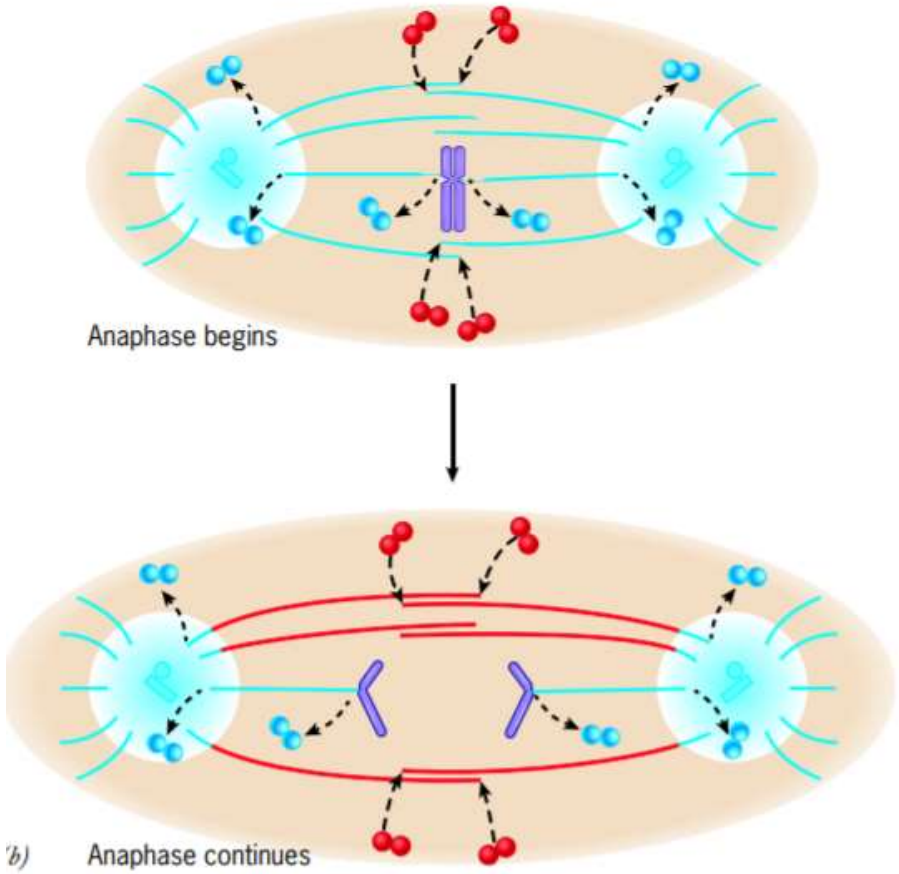
(2) the residence of several motor proteins involved in chromosome motility

(3) a key component in the signaling pathway of an important mitotic checkpoint

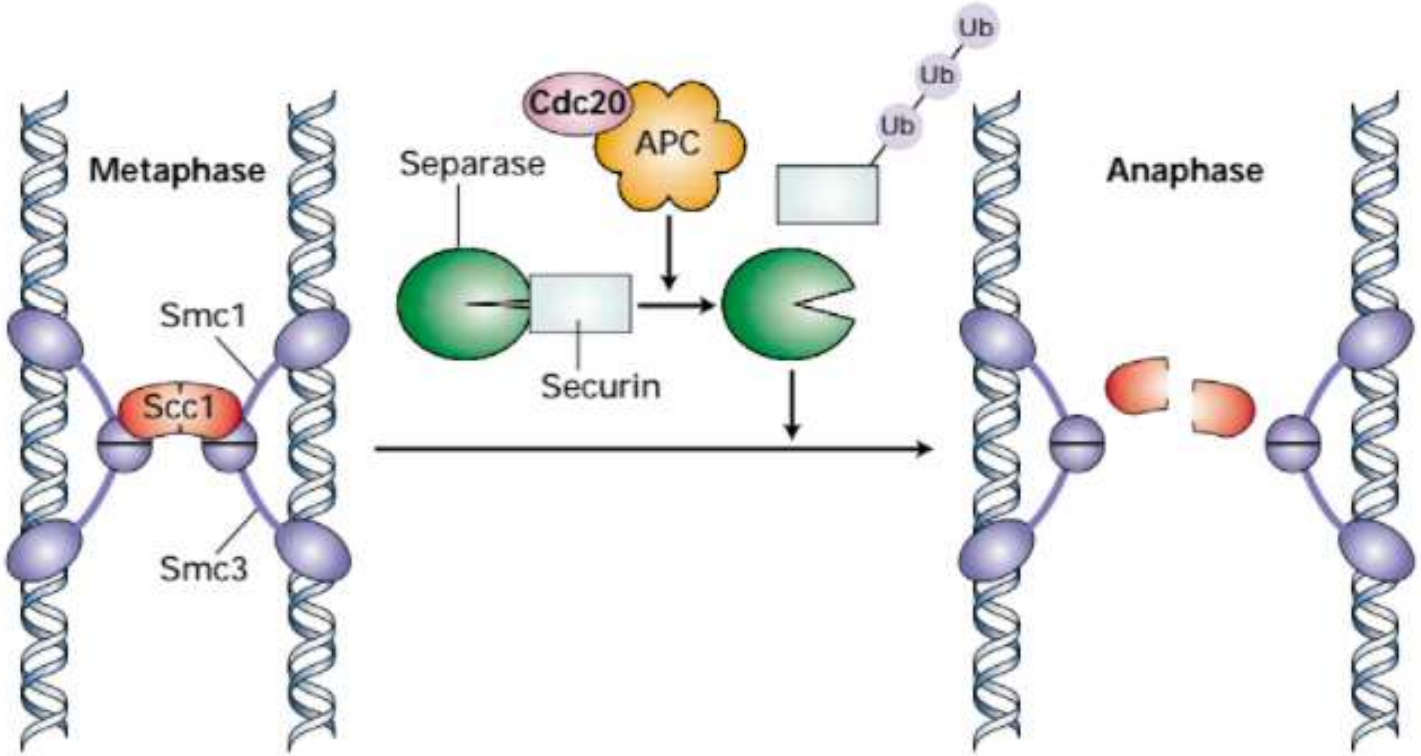


Anaphase

- The sister-chromatid cohesion depends on the **cohesin complex**, that is deposited along the chromosomes as they are duplicated in S phase.
- Separation of the **sister chromatids** occurs at the *metaphase-to-anaphase transition.*
  - Anaphase begins with a sudden disruption of the cohesion between sister chromatids
  - **M-Cdk** activity sets the stage for this event
  - The **anaphase-promoting complex (APC)** throws the switch that initiates sister-chromatid separation.

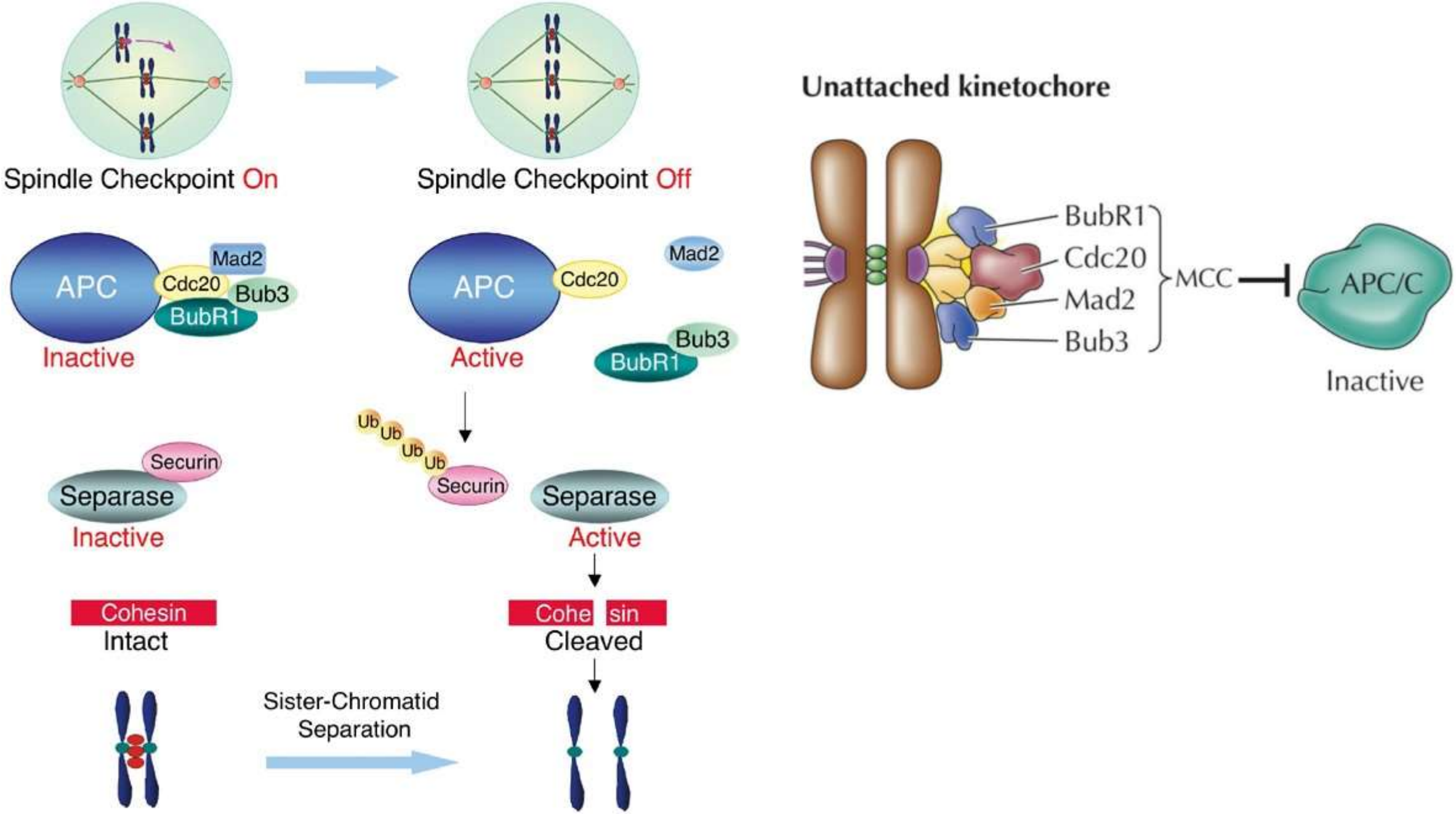


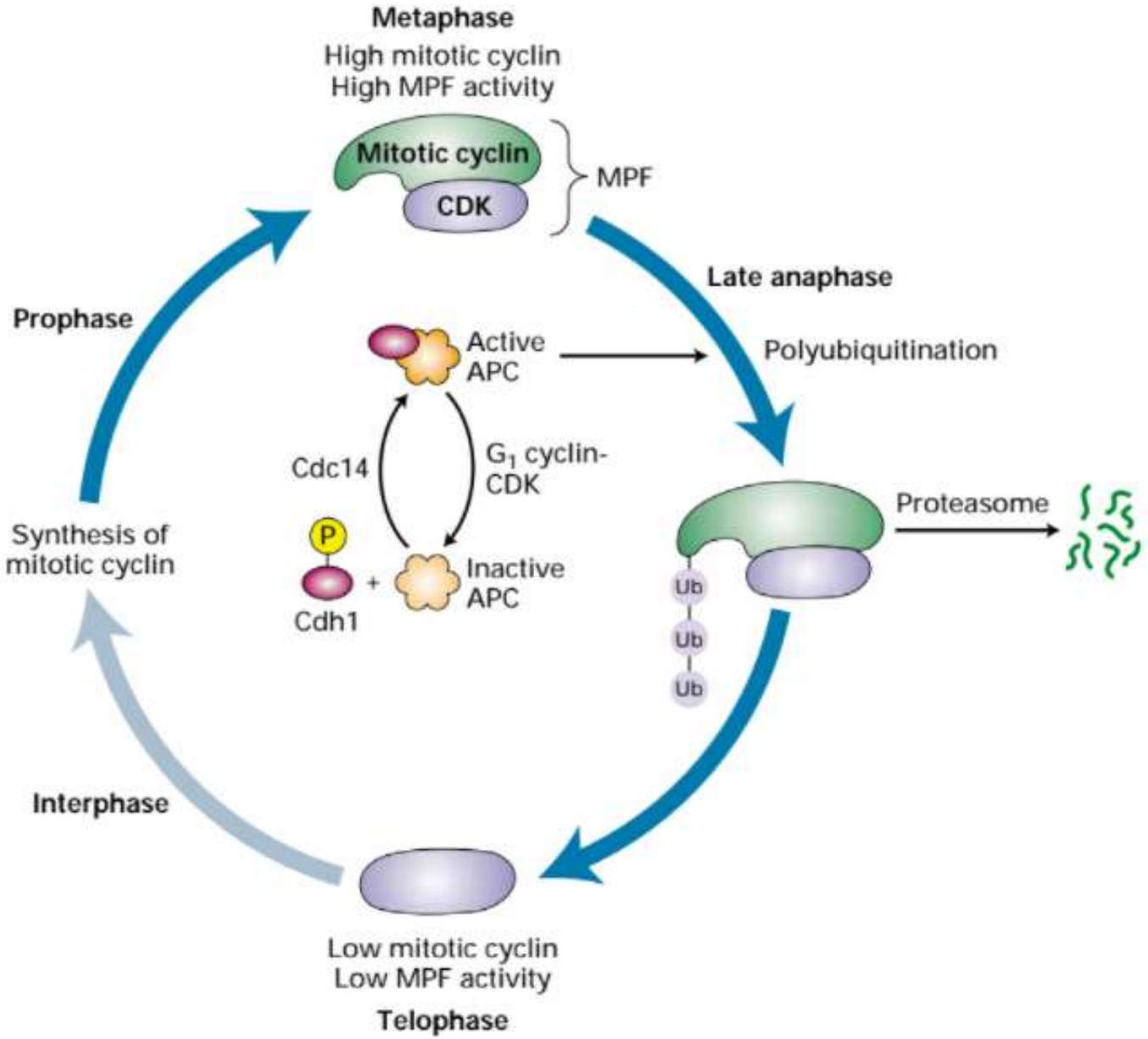
Anaphase promoting complex (APC)



The Spindle-Attachment Checkpoint

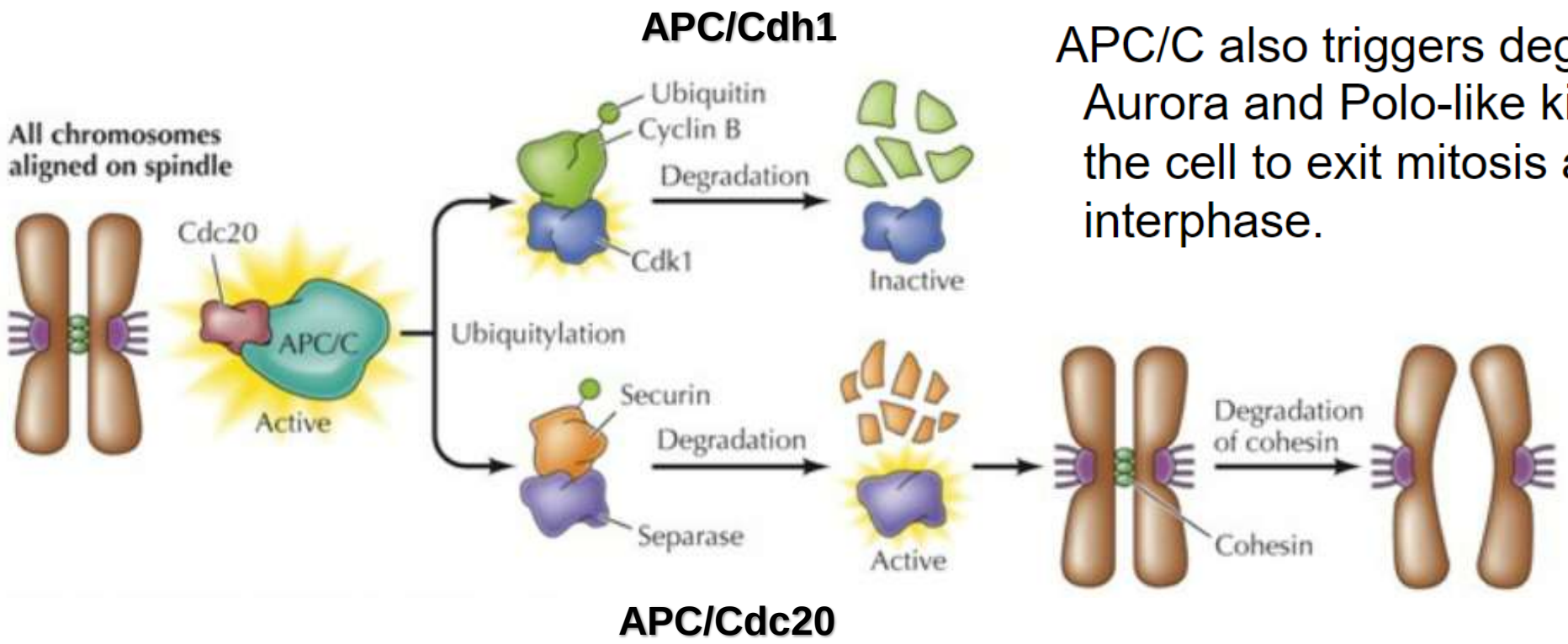
- In most cell types, before sister-chromatid separation occurs all chromosomes must be properly attached to the spindle.
- The checkpoint depends on a sensor mechanism that monitors the state of the **kinetochore** , the specialized region of the chromosome that attaches to microtubules of the spindle.
  - Any **kinetochore** that is not properly attached to the spindle sends out a negative signal to the cell-cycle control system, blocking **Cdc20-APC** activation and sister-chromatid separation.


Spindle Checkpoint On
Spindle Checkpoint Off
APC
Cdc20
Mad2
Bub3
BubR1
Inactive
APC
Cdc20
Mad2
Bub3
BubR1
Active
Ub
Ub
Ub
Ub
Securin
Securin
Separase
Inactive
Separase
Active
Cohesin
Intact
Cohe
sin
Cleaved
Sister-Chromatid
Separation
Unattached kinetochore
BubR1
Cdc20
Mad2
Bub3
MCC
APC/C
Inactive



In late anaphase

- APC activity is directed toward mitotic cyclins by a specificity factor, called Cdh1
- A specific phosphatase called Cdc14 removes the regulatory phosphate from the Cdh1 late in anaphase.



APC/C also triggers degradation of Aurora and Polo-like kinases, allowing the cell to exit mitosis and return to interphase.

Question 1

- Cells in S- phase of the cell cycle were fused to cells in the following stages of cell cycle: (a) G1 phase, (b) G2 phase, (c) M phase these cells were then grown in the medium containing tritiated thymidine. The maximal amount of freshly labelled DNA is likely to be obtained in S- phase cells fused with:

- (1) G1 phase cells
- (2) G2 phase cells
- (3) M phase cells
- (4) Both G1 and G2 phase cells

Question 2

Given below are events in the cell cycle.

(a) Phosphorylation of lamin A, B, C
(b) Phosphorylation of Rb (Retinoblastoma protein)
(c) Polyubiquitination of securin
(d) Association of inner nuclear membrane proteins

Which one of the following reflects the correct sequence of events in the mammalian cell cycle?

1. a→b→c→d
2. b→c→d→a
3. c→a→b→d
4. b→a→c→d

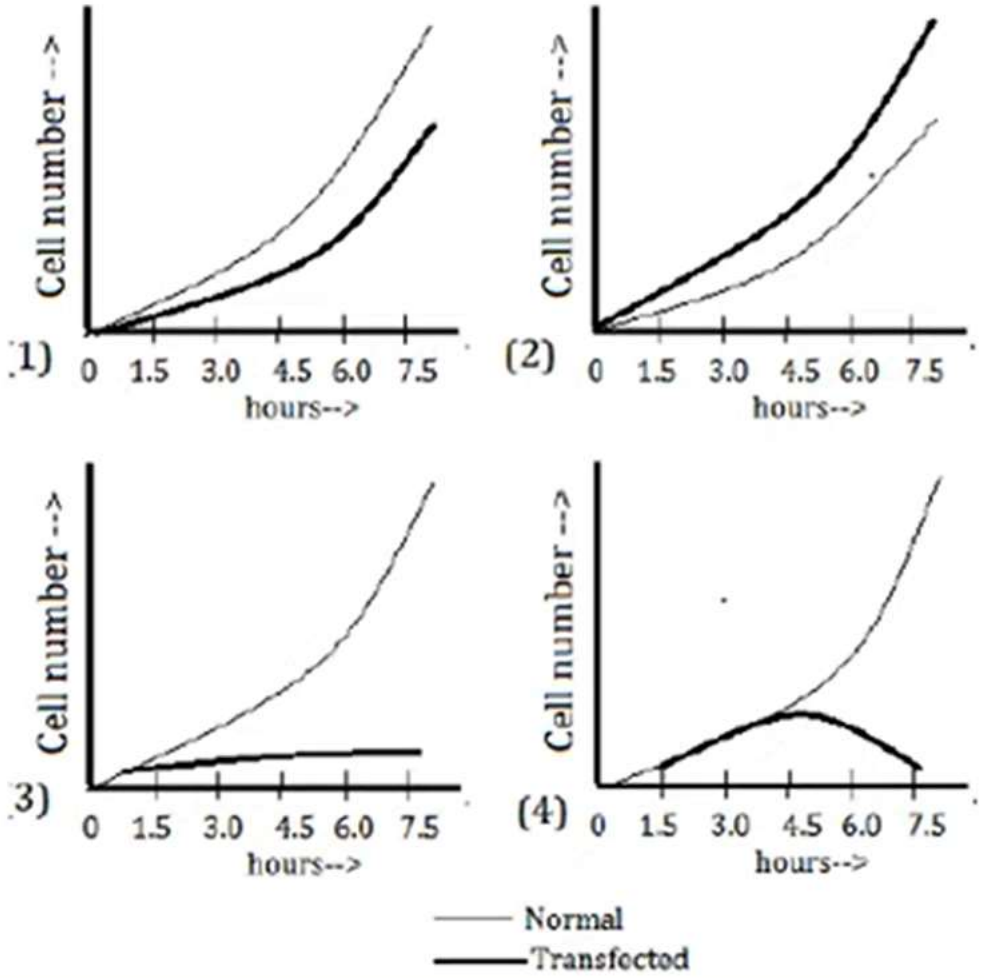
Question 3

80. Maturation-promoting factor (MPF) controls the initiation of mitosis in eukaryotic cells. MPF kinase activity requires cyclin B. Cyclin B is required for chromosome condensation and breakdown of the nuclear envelope into vesicles. Cyclin B degradation is followed by chromosome decondensation, nuclear envelope reformation and exit from mitosis. This requires ubiquitination of a cyclin destruction box motif in cyclin B. RNase-treated Xenopus egg extracts and sperm chromatin were, mixed. MPF activity increased with chromosome condensation and nuclear envelope breakdown. However, this was not followed by chromosome decondensation and nuclear envelope reformation because

1. RNase contamination persisted in the system
2. cyclin B was missing from the system
3. ubiquitin ligase had been overexpressed
4. cyclin B lacking the cyclin destruction box had been overexpressed

Question 4

83. During cell cycle regulation in eukaryotes, there are post-translational modifications of protein factors, which act as switches for different phases of cell cycle. A cell population of yeast was transfected with gene for wee 1 kinase (modifies cdc2 protein).Assuming that the transfection efficiency was 50% only, which of the following graphical representation of the results is most appropriate?



Question 5

28. Regarding microtubule assembly and disassembly during cell division, which will be the most appropriate answer?

1. Once formed, kinetochore microtubules depolymerize at the plus ends throughout mitosis.
2. Once formed, kinetochore microtubules polymerize at the plus ends throughout mitosis.
3. Kinetochore microtubules polymerize at their plus ends up to anaphase, at which point they begin to depolymerize.
4. Kinetochore microtubules polymerize at their minus ends up to cytokinesis, at which point they depolymerize.

Question 6

Q.3 During cell cycle, entry in the S-phase is tightly regulated. This is possible because:

A. APC/C promotes ubiquitination of S-phase cyclins and mitotic cyclins, marking them for proteolyses at the mitotic exit.

B. Cyclin B1 helps in the activation of S-phase CDKs only in late G1.

C. As mitotic CDK activity declines in late mitosis, cdc14 phosphatase activates APC/C by dephosphorylating Cdh1, thus promoting formation of APC/C^{Cdh1}.

D. Securin keeps S-phase cyclins in inactive state till late G1.

Which one of the options represents all correct statements?

(1) A and B

(2) A and C

(3) B and C

(4) B and D

CSI