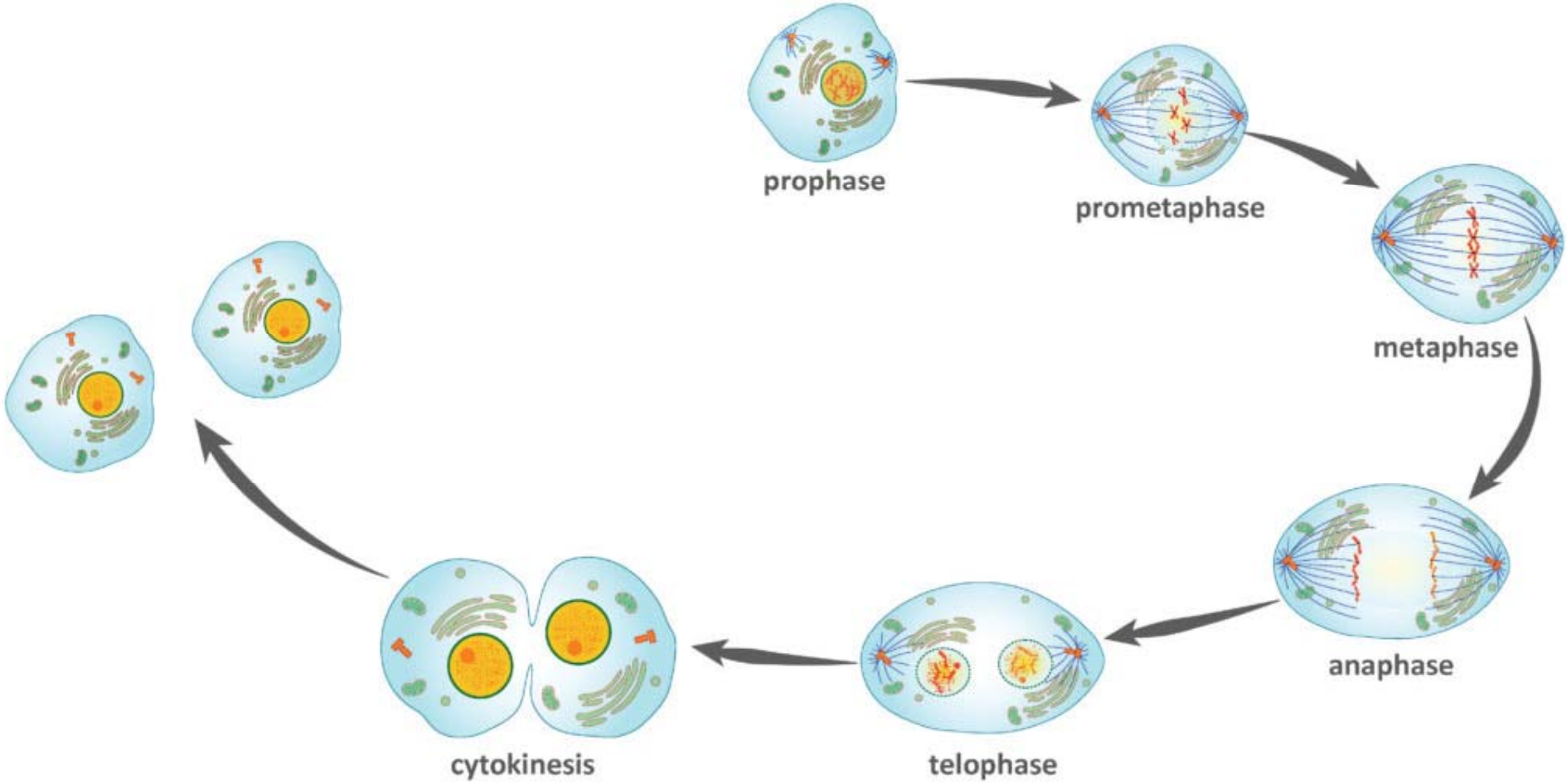
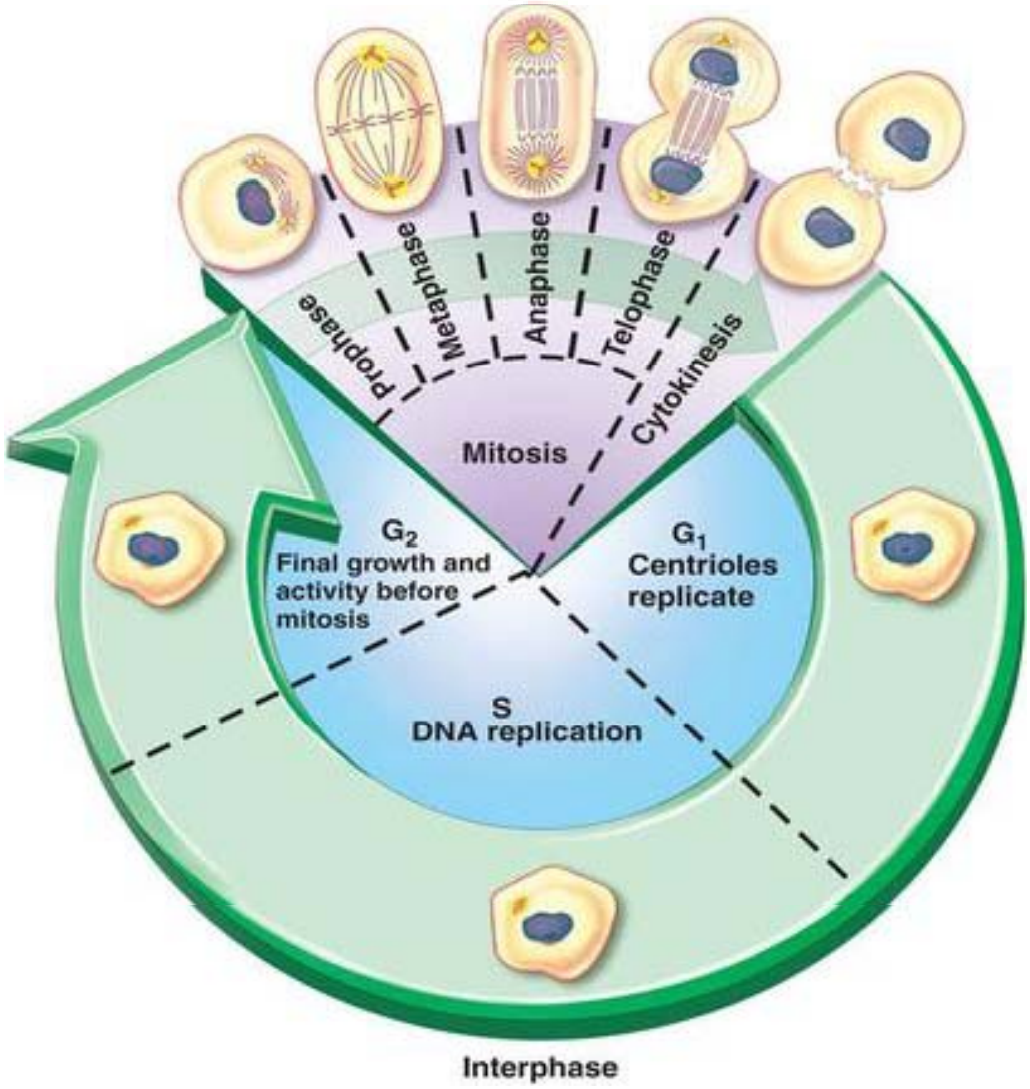
Cell Cycle Regulation

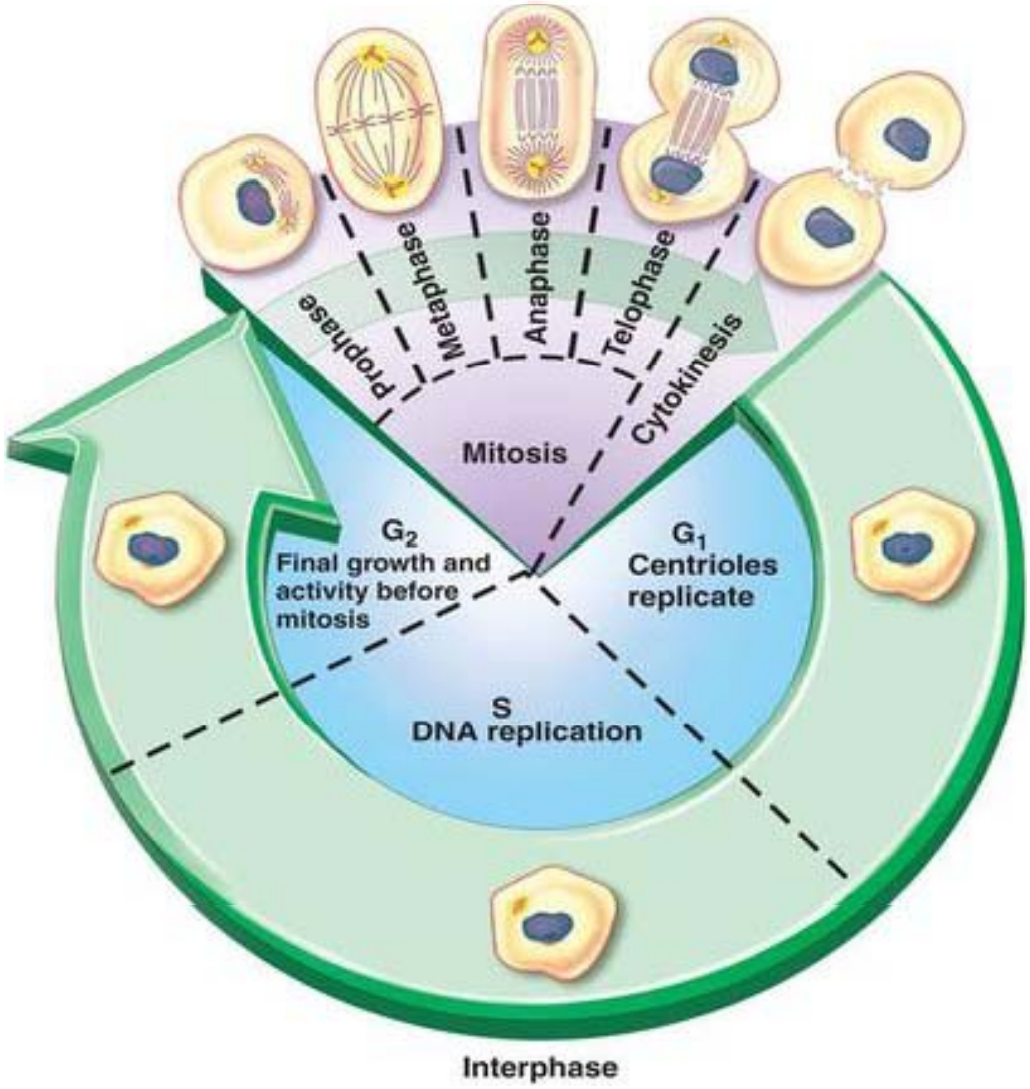
The goal of each cell division is to generate two daughter cells of identical genetic makeup.



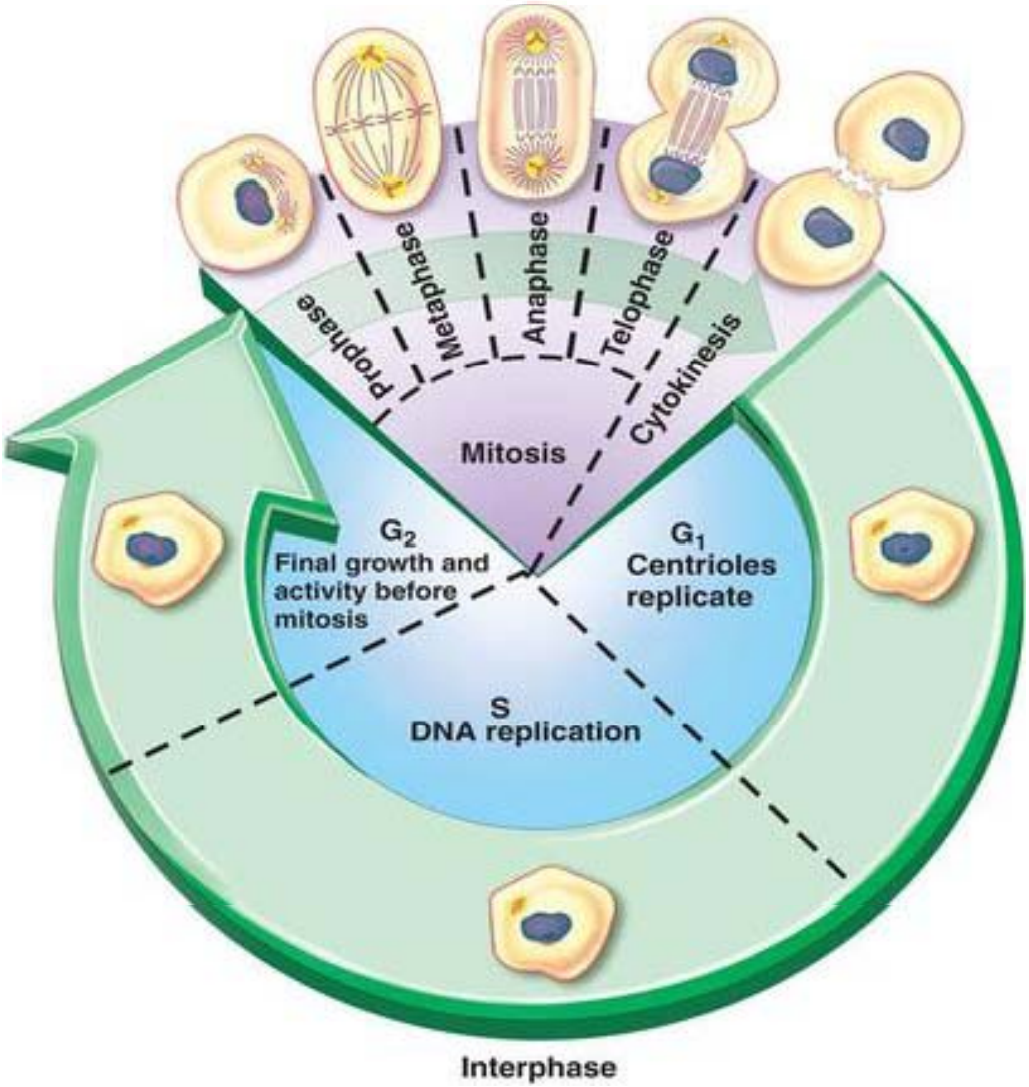
cell cycle events must occur in the proper order



High accuracy and fidelity are required to ensure that DNA replication is carried out correctly and that each daughter cell inherits the correct amount of genetic material



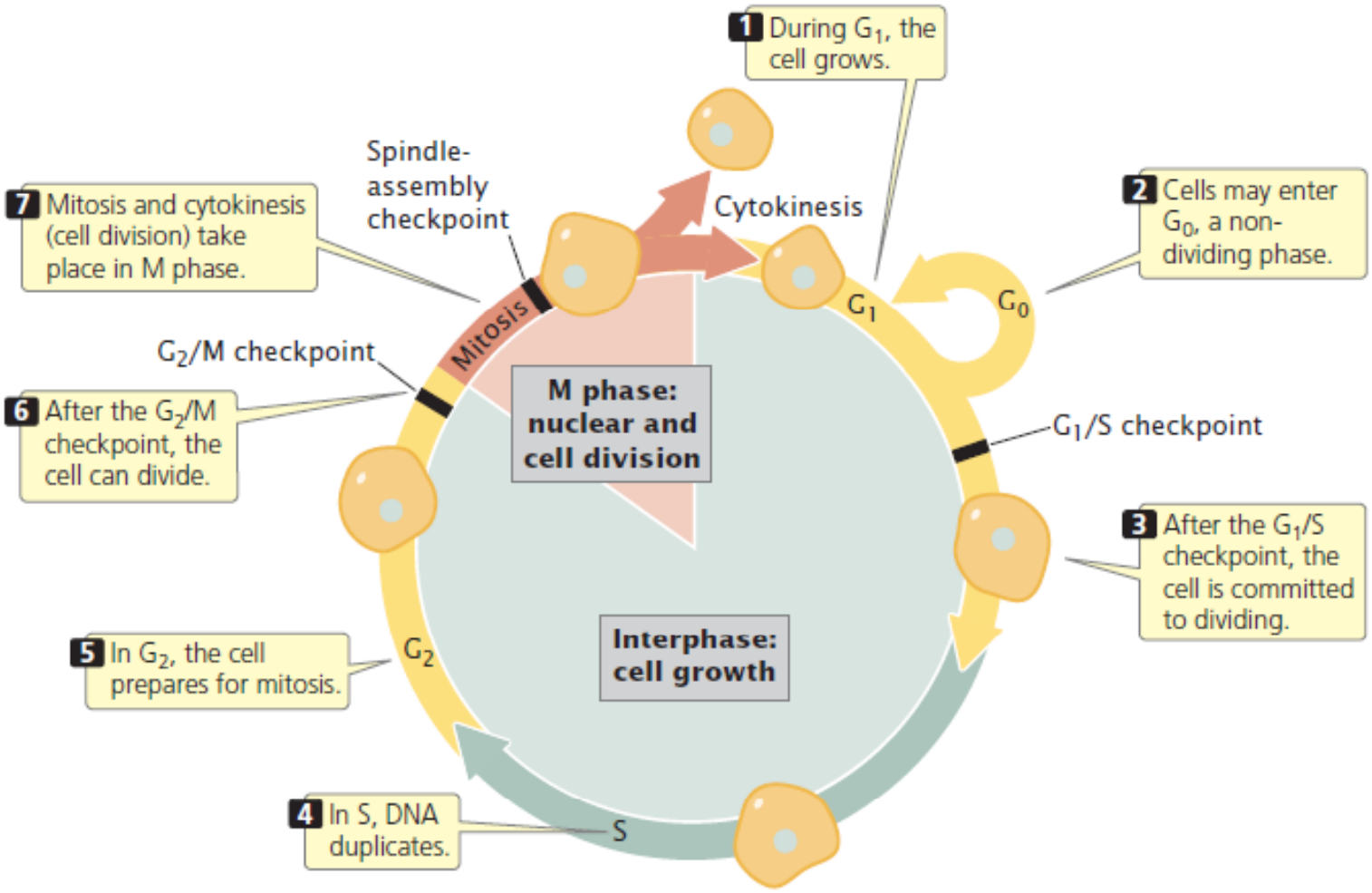
The loss of normal controls on cell division is the fundamental defect in cancer



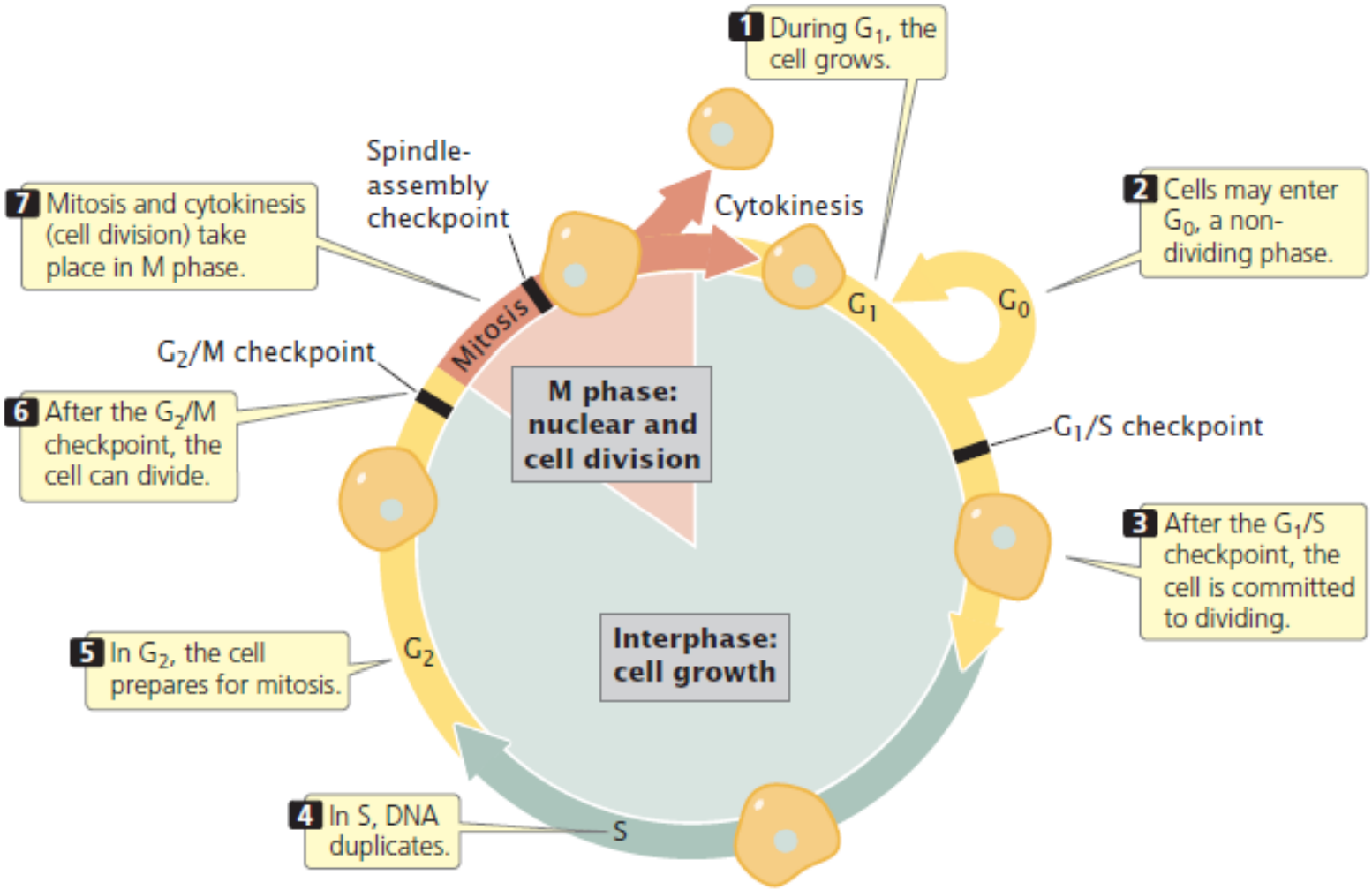
Cell Cycle - Events

four major phases

1. G1:cells grow in size and synthesize RNAs and proteins

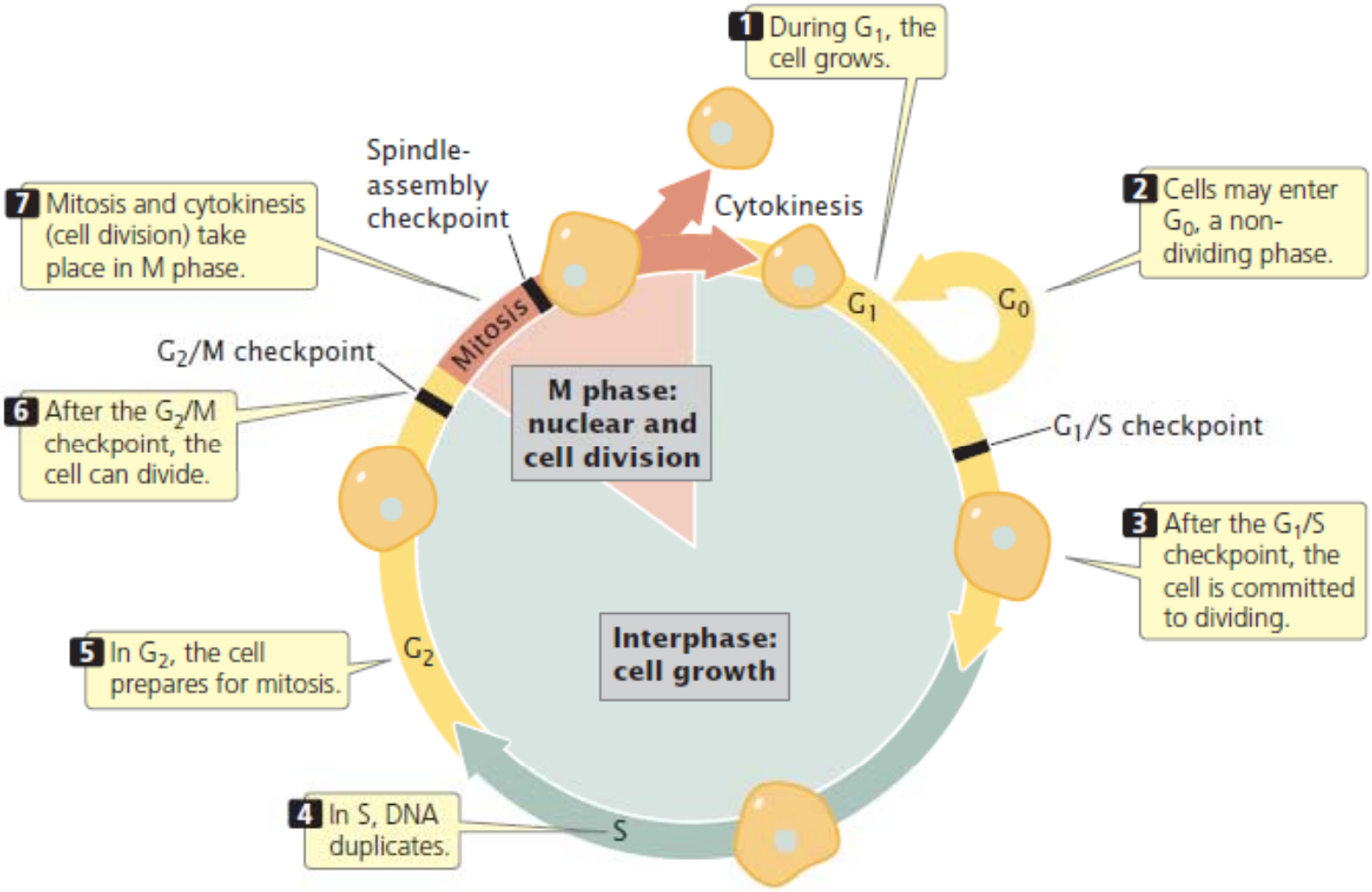


2. S: synthesis phase, the period in which cells actively replicate their chromosomes.

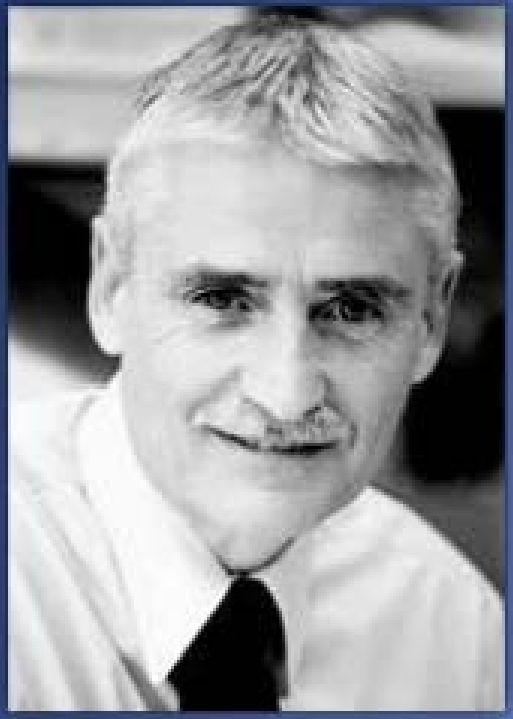


3. G2: several additional biochemical events necessary for cell division take place

4. M: mitotic phase, which is the division phase.



Cell Cycle- Regulation



Leland H. Hartwell

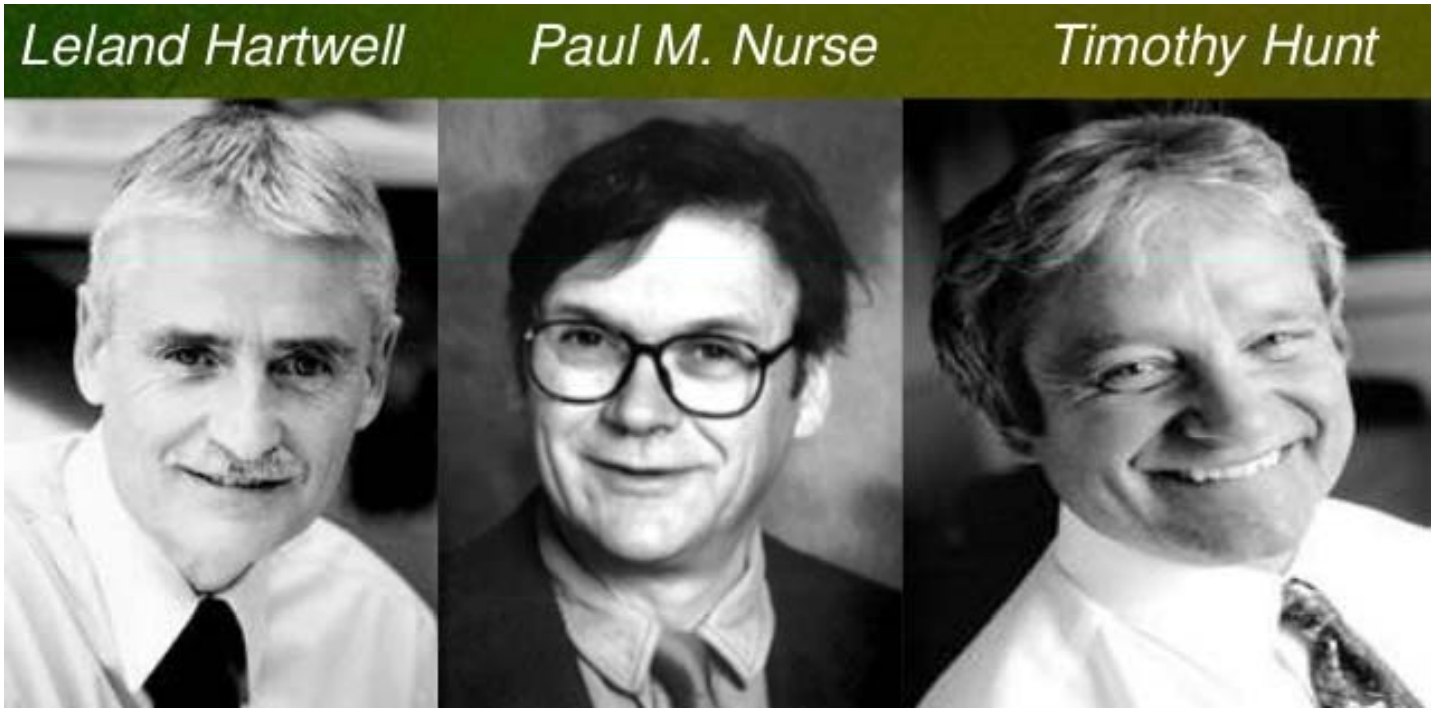
Tim Hunt

Sir Paul M. Nurse

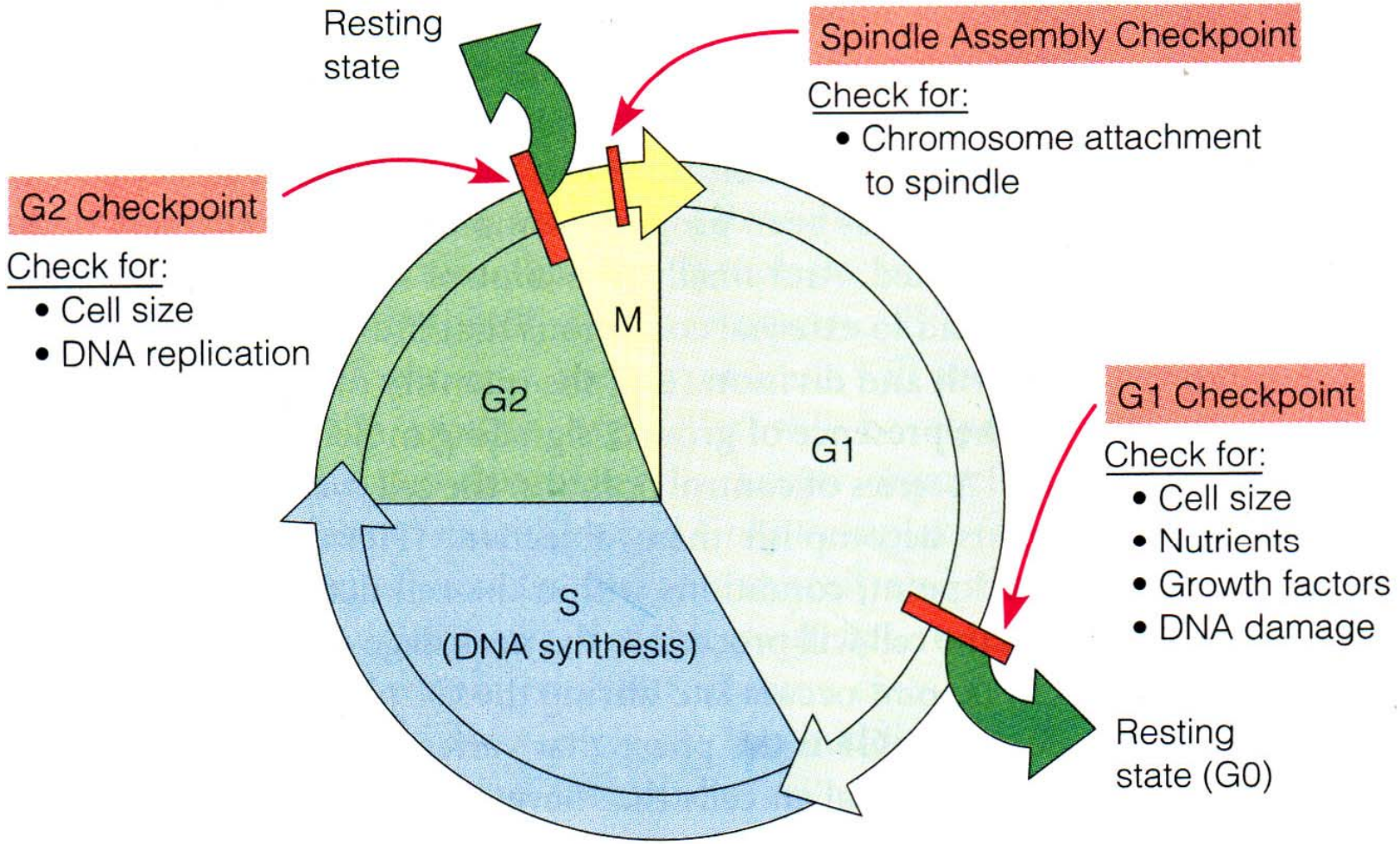
The Nobel Prize in Physiology or Medicine 2001 was awarded jointly to Leland H. Hartwell, Tim Hunt and Sir Paul M. Nurse "for their discoveries of key regulators of the cell cycle".

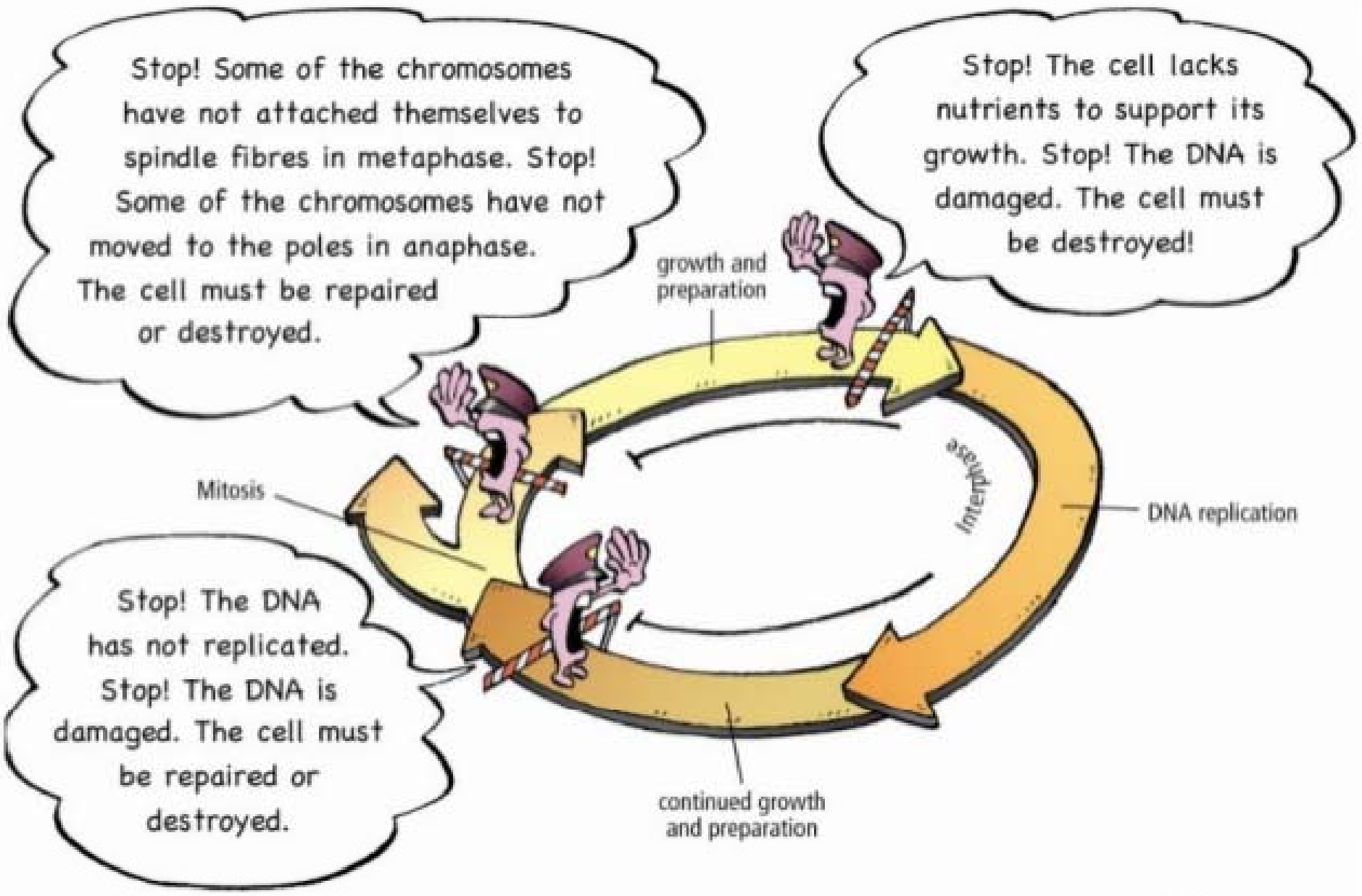
Cell Cycle – Regulation

Nobel Prize in Physiology or Medicine in 2001 for the initial experiments that elucidated the master regulators of cell division in eukaryotes.



Cell division is controlled by checkpoint pathways that prevent initiation of each step in cell division until earlier steps on which it depends have been completed and mistakes that occurred during the process have been corrected.

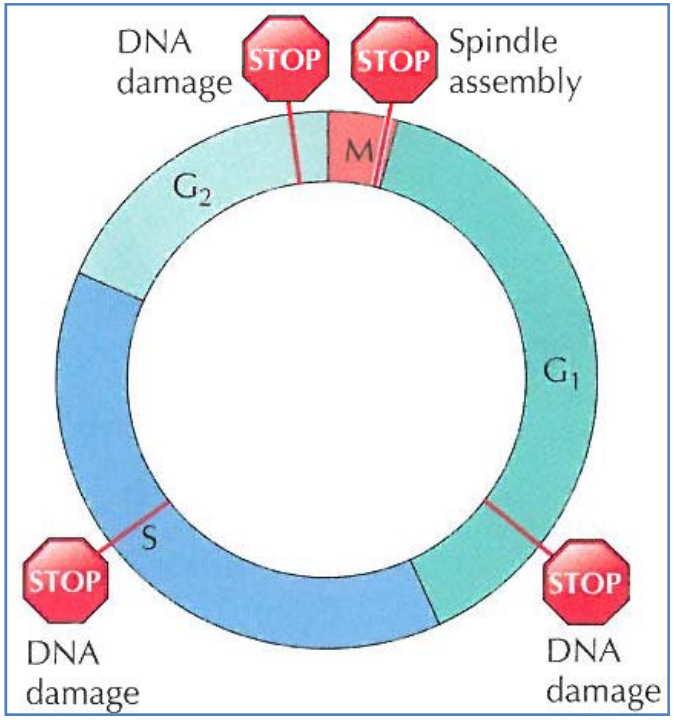



Stop! Some of the chromosomes have not attached themselves to spindle fibres in metaphase. Stop! Some of the chromosomes have not moved to the poles in anaphase. The cell must be repaired or destroyed.
Stop! The cell lacks nutrients to support its growth. Stop! The DNA is damaged. The cell must be destroyed!
growth and preparation
Mitosis
Interphase
DNA replication
Stop! The DNA has not replicated. Stop! The DNA is damaged. The cell must be repaired or destroyed.
continued growth and preparation

Cell cycle checkpoints

The coordination between different phases of the cell cycle is dependent on a series of cell cycle checkpoints that prevent entry into the next phase of the cell cycle until the events of the preceding phase have been completed.

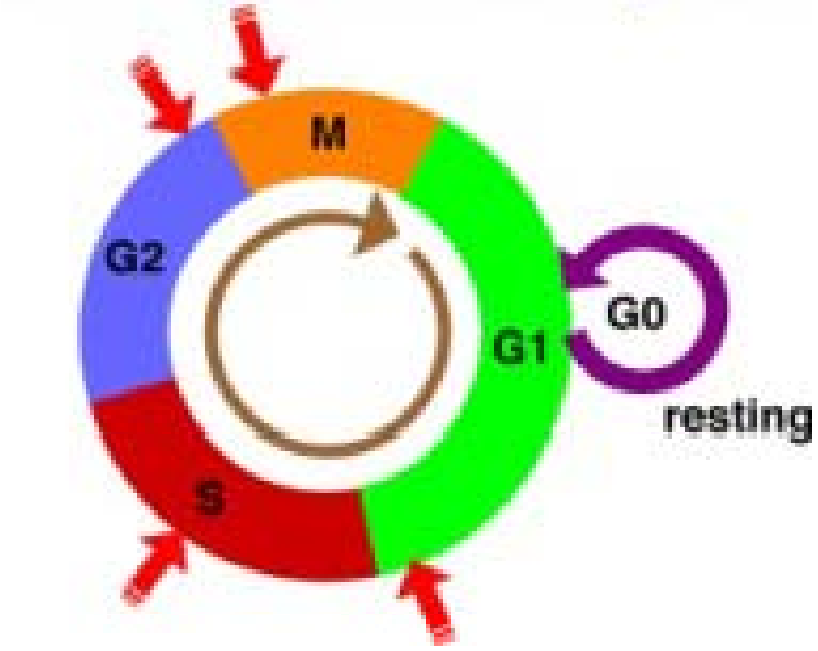
A checkpoint is a stage in the cell cycle at which the cell examines internal and external signals and decides whether or not to move forward with division.

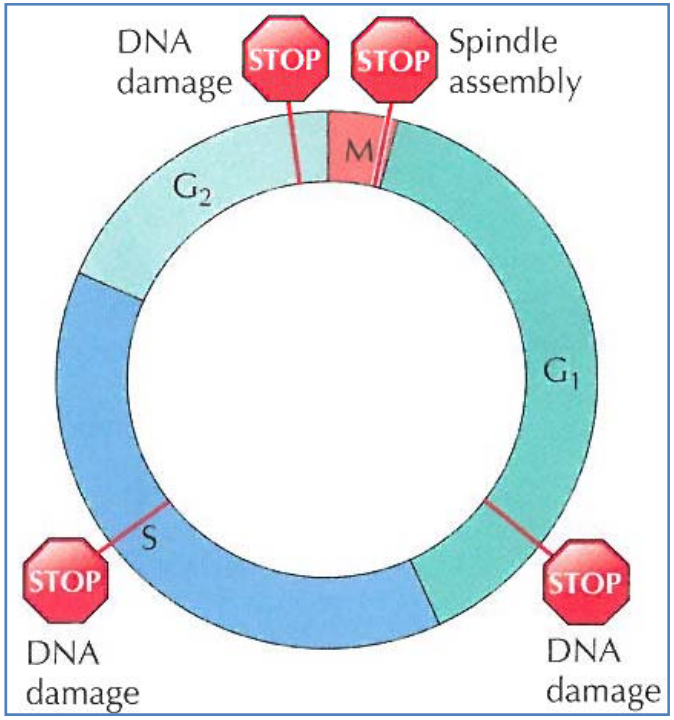


There cell cycle checkpoints are divided in to two categories:

DNA damage checkpoints and

Spindle assembly checkpoint

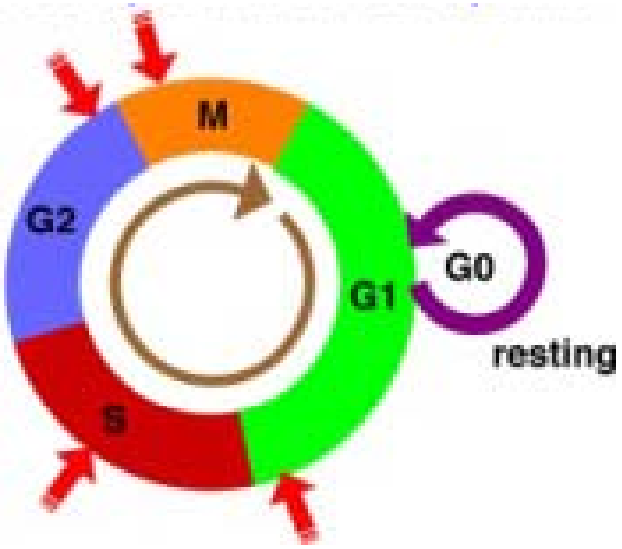


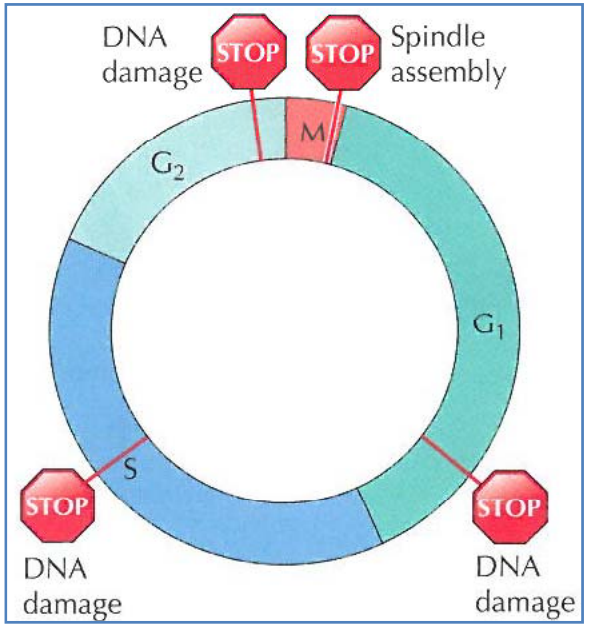


I. DNA damage checkpoints

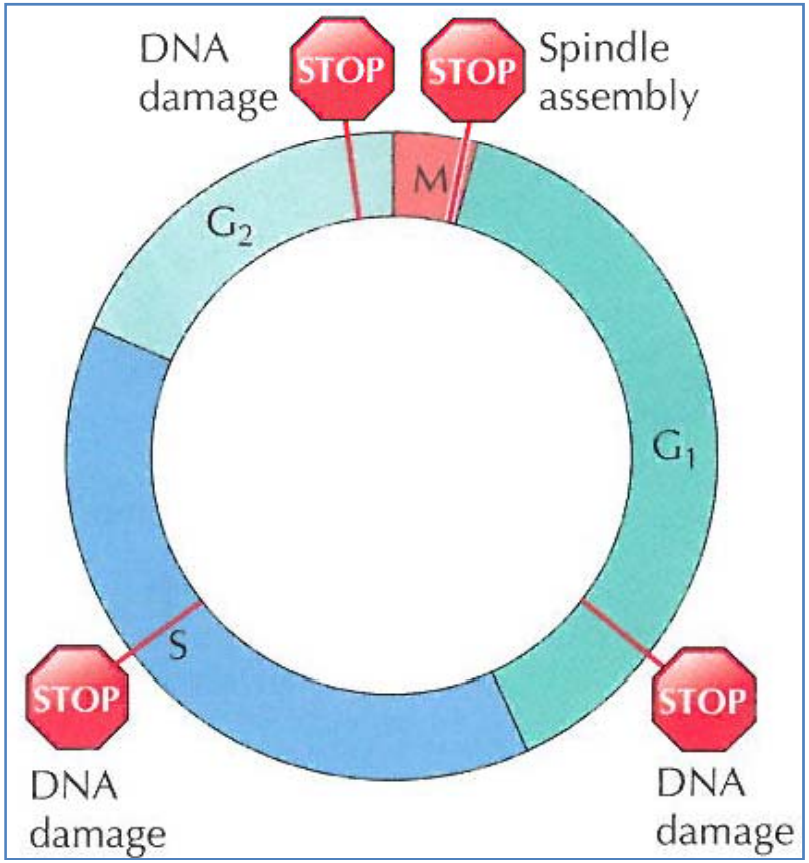
DNA damage checkpoints, function to ensure that damaged DNA is not replicated and passed on to daughter cells.

These checkpoints sense damaged or incompletely replicated DNA and coordinate further cell cycle progression with the completion of DNA replication or repair.

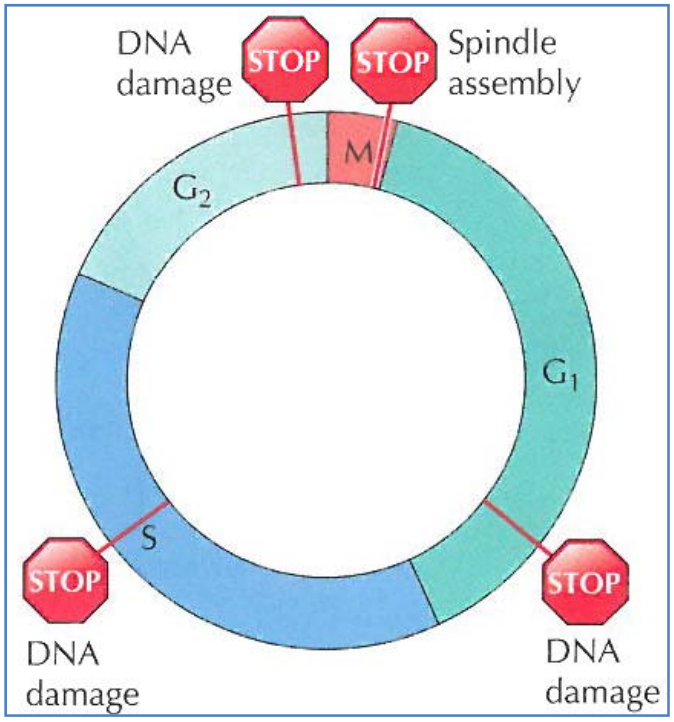




DNA damage checkpoints function in G1, S, and G2 phases of the cell cycle and named as G1 checkpoint, G2 checkpoint and S-phase checkpoint



1. G1 checkpoint: arrest at the G1checkpoint allows repair of any DNA damage to take place before the cell enters S phase, where the damaged DNA would be replicated.



at the G1/S transition

At the G1 checkpoint, a cell checks whether internal and external conditions are right for division

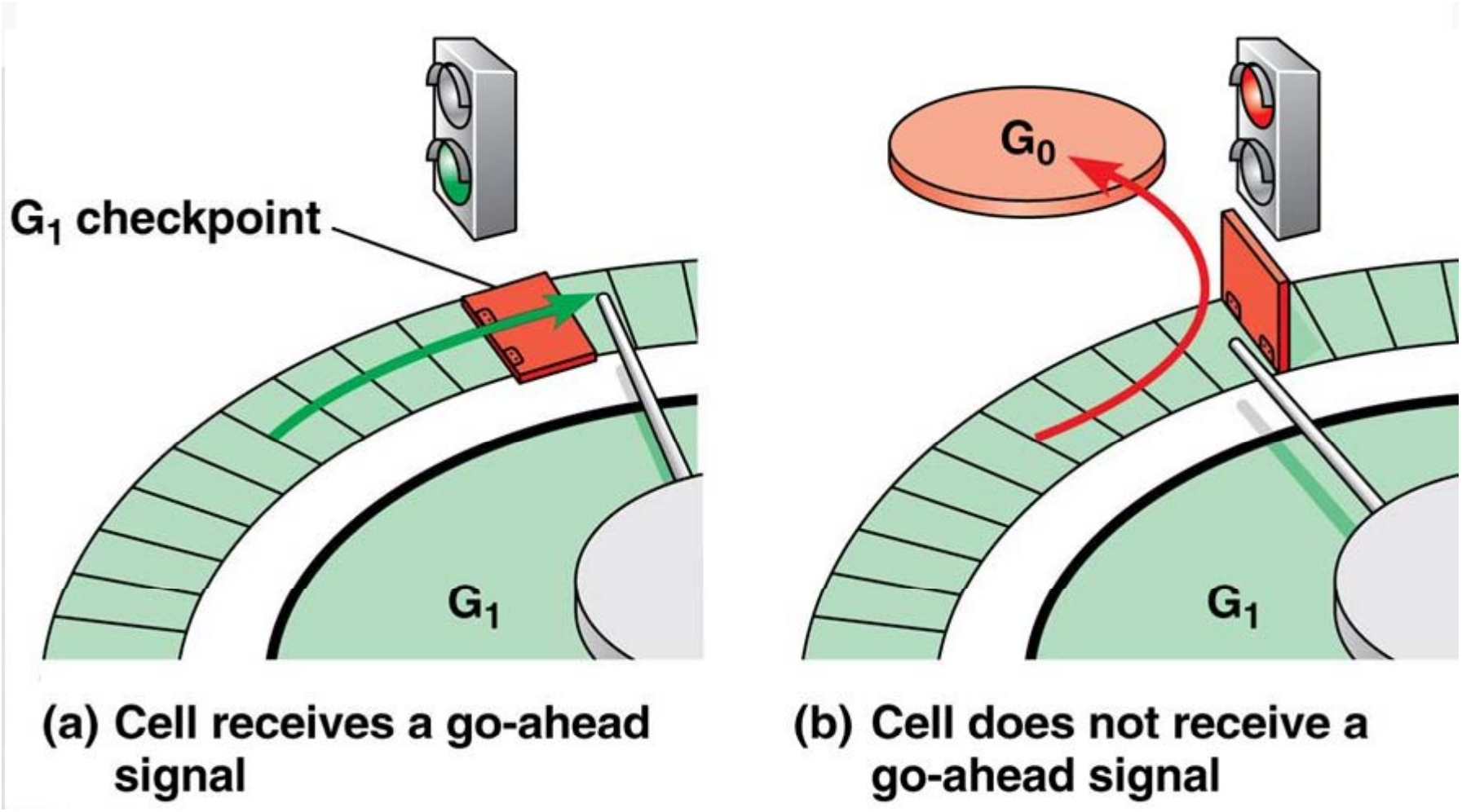
the cell might assess:

1. Size: Is the cell large enough to divide?

2. Nutrients: Does the cell have enough energy reserves or available nutrients to divide?

3. Molecular signals: Is the cell receiving positive cues (such as growth factors) from neighbors?

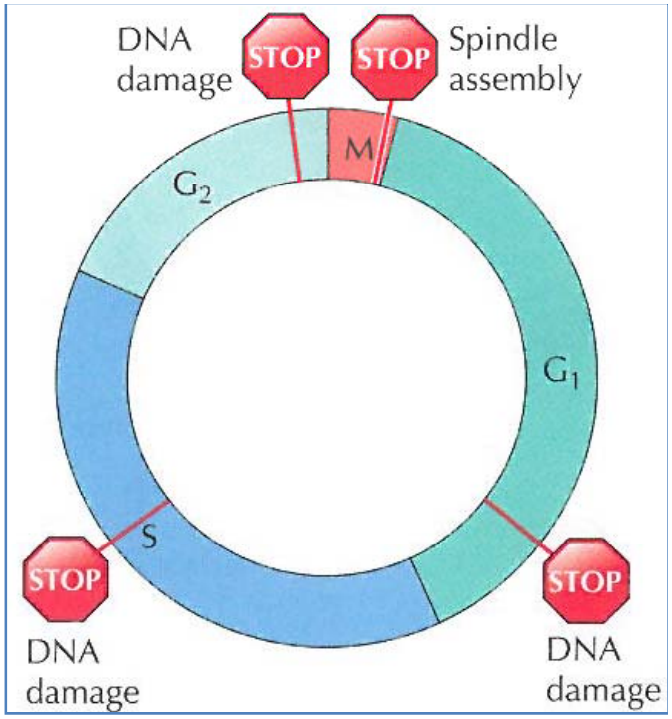
4. DNA integrity. Is any of the DNA damaged?



(a) Cell receives a go-ahead signal

(b) Cell does not receive a go-ahead signal

2. S-phase checkpoint:



The S-phase checkpoint; at S phase

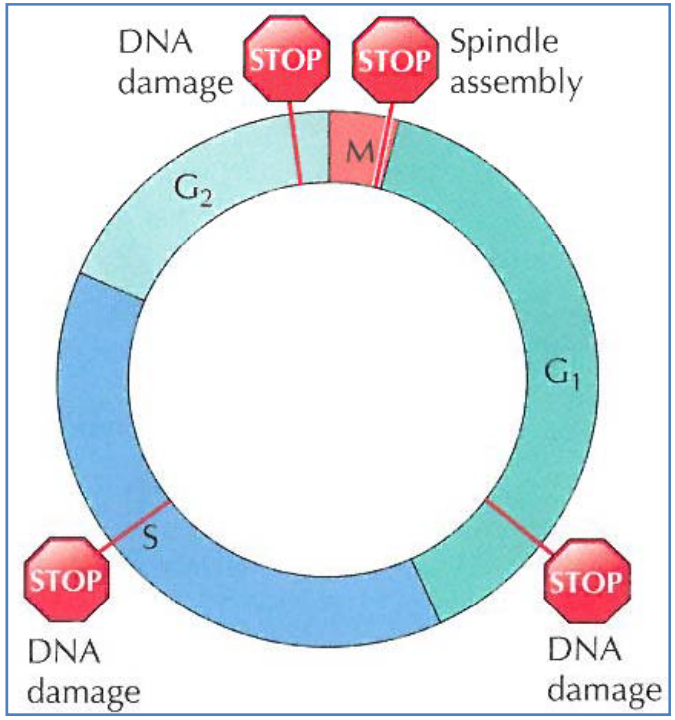
- provides continual monitoring of the integrity of DNA to ensure that damaged DNA is repaired before it is replicated,

- 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 DNA segments

2. G2 checkpoint:

G2 checkpoint prevents the initiation of mitosis if the cell contains DNA that has not been completely replicated or contains unrepaired lesions.

Such damaged DNA activates a signaling pathway that leads to cell cycle arrest.



at the G2/M transition

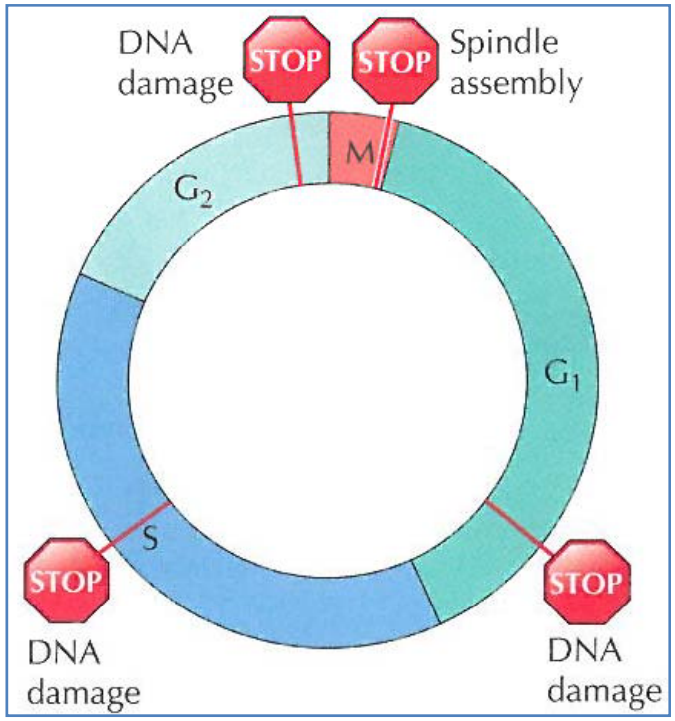
Operation of the G2 checkpoint therefore prevents the initiation of M phase until the genome has been completely replicated and any damage repaired.

Only then is the inhibition of G2 progression relieved, allowing the cell to initiate mitosis and distribute the completely replicated chromosomes to daughter cells.

the cell might assess:

1. DNA integrity: Is any of the DNA damaged?

2. DNA replication: Was the DNA completely copied during S phase?

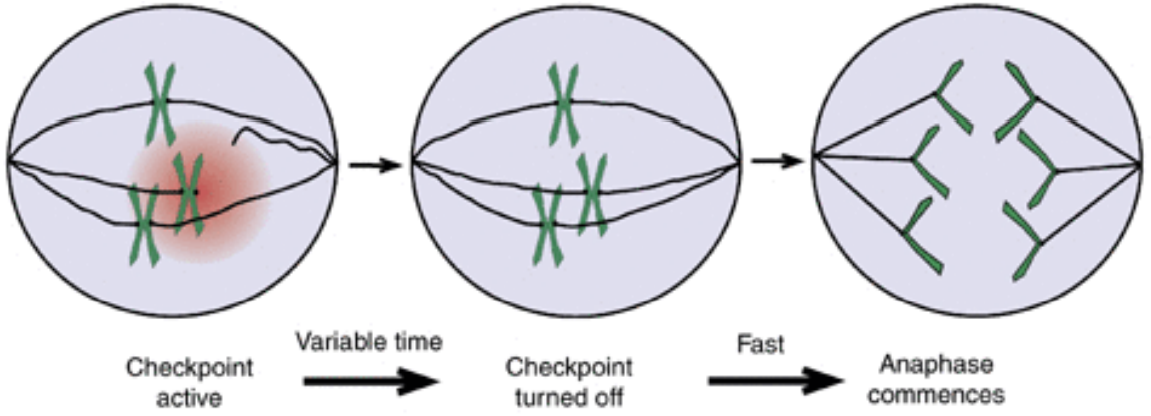


II. Spindle assembly checkpoint:

Spindle assembly checkpoint maintains the integrity of the genome occurs toward the end of mitosis.

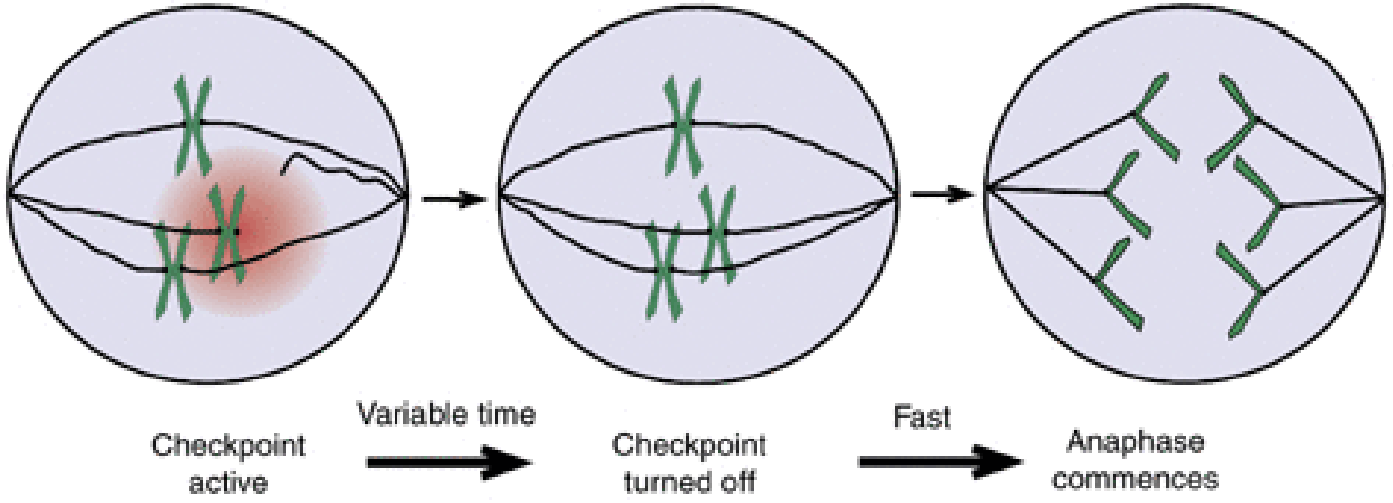
This checkpoint monitors the alignment of chromosomes on the mitotic spindle, thus ensuring that a complete set of chromosomes is distributed accurately to the daughter cells.

at the transition from metaphase to anaphase



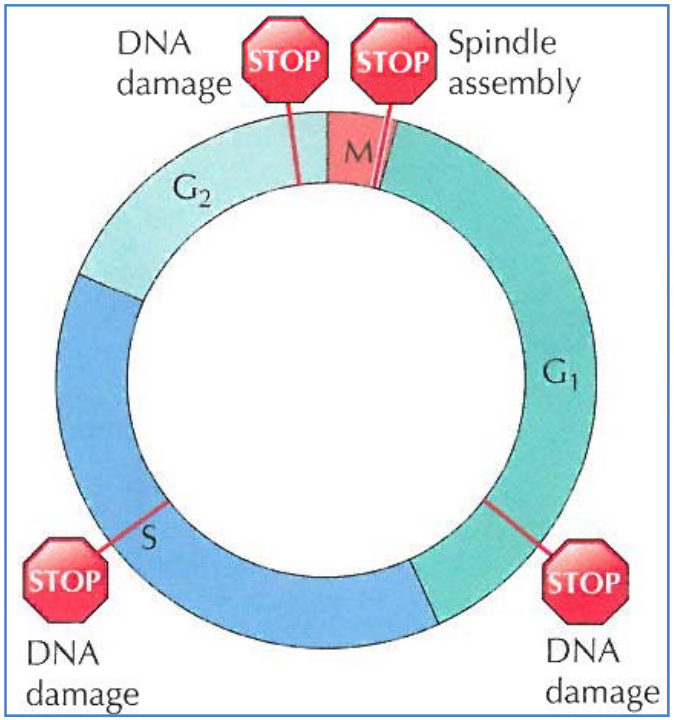
The failure of one or more chromosomes to align properly on the spindle causes mitosis to arrest at metaphase, prior to the segregation of the newly replicated chromosomes to daughter nuclei.

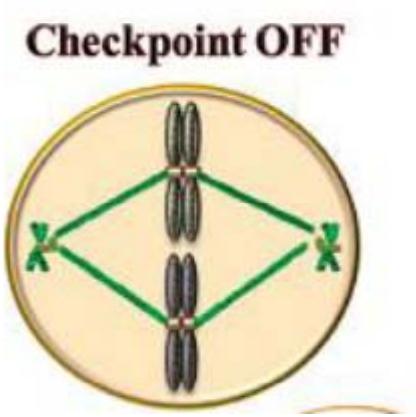
As a result of the spindle assembly checkpoint, the chromosomes do not separate until a complete complement of chromosomes has been organized for distribution to each daughter cell.

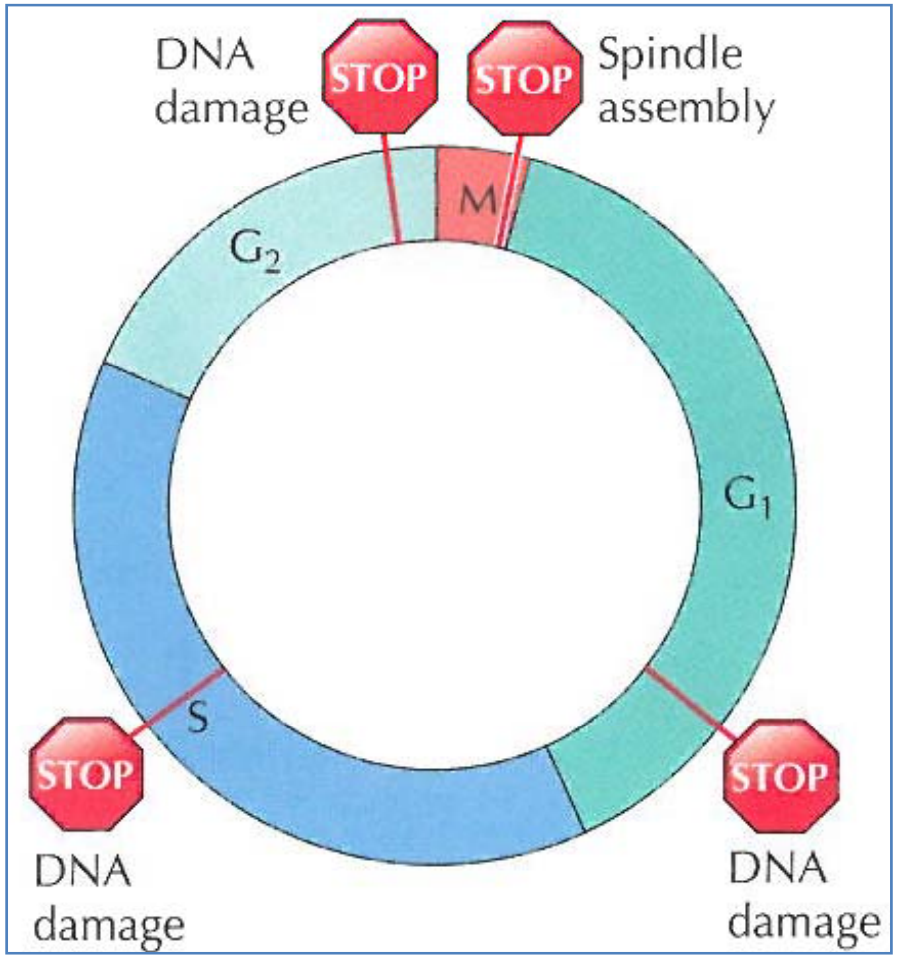


Cell might assess:

whether all the chromosomes are correctly attached to the spindle microtubules






DNA
damage
STOP
STOP
Spindle
assembly
M
G2
G1
S
STOP
STOP
DNA
damage
DNA
damage

WORKING MECHANISM OF CHECKPOINTS

Master controllers of the cell cycle

The master controllers of the cell cycle are a small number of heterodimeric proteins with kinase activity

The heterodimeric protein consists of two subunits:

1. a regulatory subunit – Cyclin
2. a catalytic subunit - Cyclin-Dependent Kinase (CDK)

Passage through each stage of cell cycle is controlled by these heterodimeric protein kinases

Function of Cyclin and CDK

These heterodimeric kinases regulate the activities of multiple proteins involved incell cycle by phosphorylating them at specific regulatory sites, activating some and inhibiting others to coordinate their activities.

TABLE 19-1	Cyclins and CDKs: Nomenclature and Their Roles in the Mammalian Cell Cycle		
CDK	Cyclin	Function	General Name
CDK1	Cyclin A, cyclin B	Mitosis	Mitotic CDKs
CDK2	Cyclin E, cyclin A	Entry into the cell cycle S phase	G_1/S phase CDKs S phase CDKs
CDK4	Cyclin D	G_1 Entry into the cell cycle	G_1 CDKs
CDK6	Cyclin D	G_1 Entry into the cell cycle	G_1 CDKs

Four types of CDKs : CDK1, CDK2, CDK4, and CDK6

Four types of Cyclins: Cyclin A, Cyclin B, Cyclin D and Cyclin E

The concentrations of the catalytic subunits (CDKs), are constant throughout the cell cycle.

However, they have no kinase activity unless they are associated with a regulatory cyclin subunit.

Each CDK can associate with one or two different cyclins that determine the substrate specificity of the complex, that is, which proteins it phosphorylates.

CDK	Cyclin	Function	General Name
CDK1	Cyclin A, cyclin B	Mitosis	Mitotic CDKs
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CDK4	Cyclin D	G_1 Entry into the cell cycle	G_1 CDKs
CDK6	Cyclin D	G_1 Entry into the cell cycle	G_1 CDKs

Each cyclin is only present and active during the cell cycle stage it promotes and hence restricts the kinase activity of the CDKs it binds to just that cell cycle stage.

Cyclin-CDK complexes activate or inhibit hundreds of proteins involved in cell cycle progression by phosphorylating them at specific regulatory sites.

TABLE 19-1 Cyclins and CDKs: Nomenclature and Their Roles in the Mammalian Cell Cycle

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CDK6	Cyclin D	G_1 Entry into the cell cycle	G_1 CDKs

Thus proper progression through the cell cycle is governed by activation of the appropriate cyclin-CDK complex at the appropriate time

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CDK6	Cyclin D	G_1 Entry into the cell cycle	G_1 CDKs

The activity of the CDKs fluctuates during the cell cycle.

The oscillations in CDK activity are a fundamental aspect of eukaryotic cell cycle control

Laid on the cell cycle oscillator machinery is a system of surveillance mechanisms that ensures that the next cell cycle event is not activated before the preceding one has been completed or before errors that occurred during the preceding step are corrected.

These surveillance mechanisms are called checkpoint pathways

In addition to the cyclin-CDK activity, regulated degradation of proteins also plays a prominent role in important cell cycle transitions.

Since protein degradation is irreversible, this ensures that the processes move in only one direction through the cell cycle.

These multiple layers of control put on the cell cycle control machinery ensure that the cell cycle is robust and error free.

Cyclin-Dependent Kinases (CDKs)

Cyclin-dependent kinases are small (30-40 kD) serine/threonine kinases.

They are not active in the monomeric form, but require an activating subunit to be active as a protein kinase.

Its activity is specified by cell-cycle stage specific cyclin subunits.

Mammalian cells contain as many as nine CDKs, with four of them, CDK1, CDK2, CDK4, and CDK6, having clearly been shown to regulate cell cycle progression.

They bind to different types of cyclins and together promote different cell cycle transitions.

- CDK4 and CDK6 are G1 CDKs and promote entry into the cell cycle
- CDK2 functions as a G1/S phase, S phase and G2 phahe CDK, and
- CDK1 is the mitotic CDK

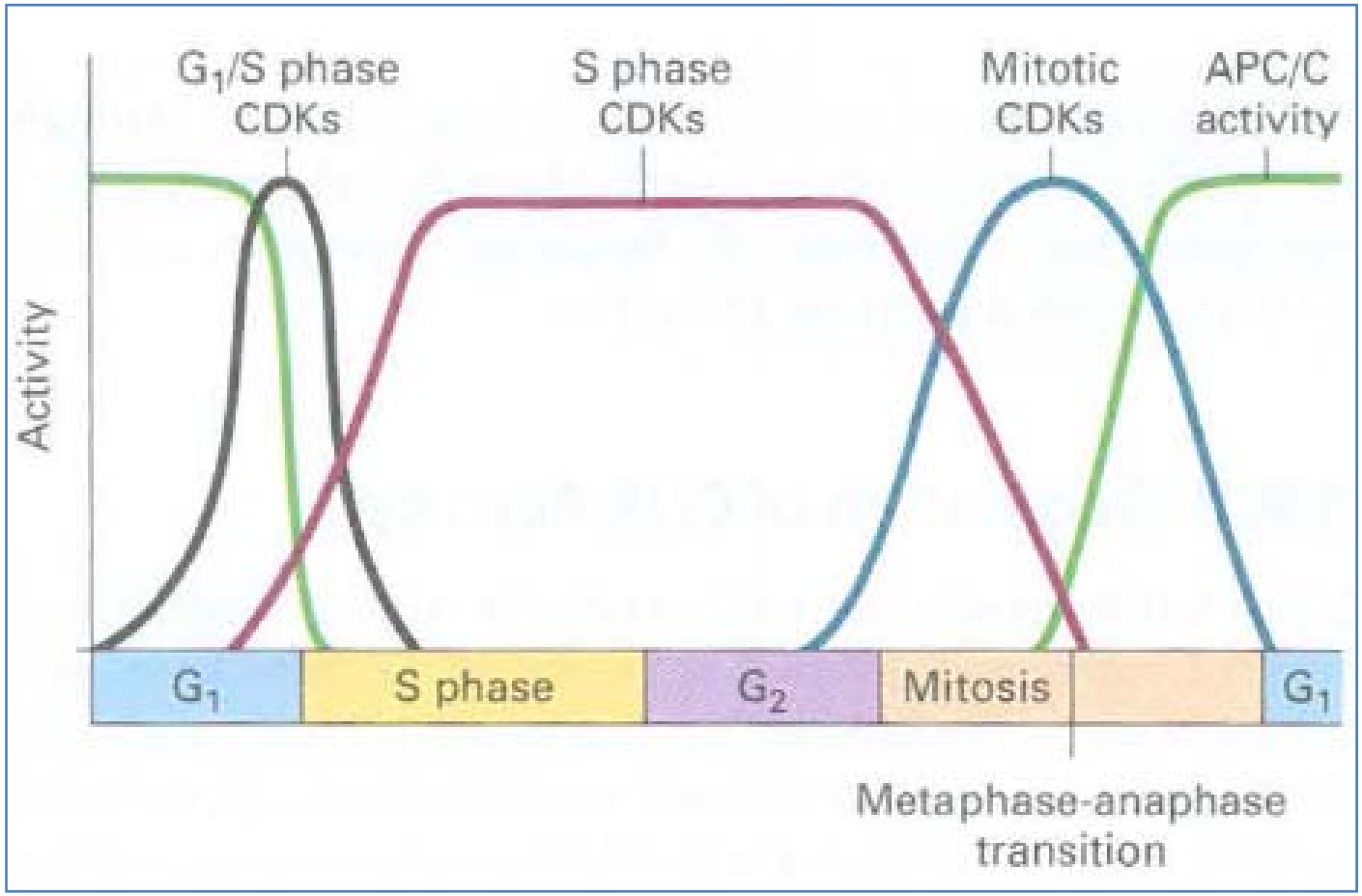
CDK	General Name
CDK1	Mitotic CDKs
CDK2	G_1/S phase CDKs S phase CDKs
CDK4	G_1 CDKs
CDK6	G_1 CDKs

Three key features CDK

1. Cyclin-dependent kinases (CDKs) are only active when bound to a regulatory cyclin subunit.

2. Different types of cyclin-CDK complexes initiate different events. G1 CDKs and G1/S phase CDKs promote entry into the cell cycle, S phase CDKs trigger S phase, and mitotic CDKs initiate the events of mitosis.

3. Multiple mechanisms are in place to ensure that the different CDKs are only active in the stages of the cell cycle they trigger. CDKs are not only regulated by cyclin binding but also by activating and inhibitory phosphorylation. Together, these regulatory events ensure that CDKs are only active at the appropriate cell cycle stage.


G1/S phase CDKs
S phase CDKs
Mitotic CDKs
APC/C activity
Activity
G1
S phase
G2
Mitosis
G1
Metaphase-anaphase transition

Cyclins

Cyclins Determine the Activity of CDKs (named because their levels change during the cell cycle)

Cyclins are divided into four classes defined by their presence and activity during the cell cycle: G1 cyclins, G1/S cyclins, S phase cyclins, and mitotic cyclins.

The different types of cyclins are quite distinct from each other in protein sequence, but all of them contain a conserved region known as the cyclin box and possess similar three-dimensional structures

three key features of Cyclins:

1. Cyclins bind to and activate CDKs. The activity and substrate specificity of any given CDK is primarily defined by the particular cyclin to which it is bound.

2. Cyclins are only present during the cell cycle stage that they trigger and are absent in other cell cycle stages.

3. Cyclins not only regulate a particular cell cycle stage but also set in motion a series of events in preparation for the next cell cycle stage. In this way, they propel the cell cycle forward.

1. G1 cyclins (Cyclin D)

The G1 cyclins coordinates the cell cycle with extracellular events.

Their activity is subject to regulation by signal transduction pathways that sense the presence of growth factors or cell proliferation inhibitory signals.

G1 cyclins (cyclin D) interacts with CDK4 and CDK6 and promote the entry in to the cell cycle.

TABLE 19-1 Cyclins and CDKs: Nomenclature and Their Roles in the Mammalian Cell Cycle

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CDK4	Cyclin D	G_1 Entry into the cell cycle	G_1 CDKs
CDK6	Cyclin D	G_1 Entry into the cell cycle	G_1 CDKs

2. G1/S cyclin (Cyclin E)

The G1/S cyclins accumulate during late G1, reach peak levels when cells enter S phase, and decline during S phase.

They are known as cyclin E and bind to CDK2.

The main function for cyclinE-CDK2 complex, together with cyclinD-CDK4/6, is to trigger the G1-S phase transition.

This transition is known as START and is defined as the point at which cells are irreversibly committed to cell division and can no longer return to the G1 state.

TABLE 19-1 Cyclins and CDKs: Nomenclature and Their Roles in the Mammalian Cell Cycle

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CDK4	Cyclin D	G_1 Entry into the cell cycle	G_1 CDKs
CDK6	Cyclin D	G_1 Entry into the cell cycle	G_1 CDKs

3. S phase cyclins (Cyclin A and Cyclin E)

S phase cyclins are synthesized at the end of G1, levels remain high throughout S phase and do not decline until early mitosis.

Two types of S phase cyclins trigger S phase in: cyclin E, which can also promote entry into the cell cycle and is therefore also a G1/S cyclin, and cyclin A.

Both cyclins bind CDK2 and are directly responsible for DNA synthesis.

TABLE 19-1 Cyclins and CDKs: Nomenclature and Their Roles in the Mammalian Cell Cycle

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CDK4	Cyclin D	G_1 Entry into the cell cycle	G_1 CDKs
CDK6	Cyclin D	G_1 Entry into the cell cycle	G_1 CDKs

4. Mitotic cyclins: Mitotic cyclins bind CDK1 to promote entry into and progression through mitosis.

The mitotic cyclins are cyclin A and cyclin B

Mitotic cyclin-CDK complexes are synthesized during S phase and G2, but their activities are held in check until DNA synthesis is completed.

TABLE 19-1 Cyclins and CDKs: Nomenclature and Their Roles in the Mammalian Cell Cycle

CDK	Cyclin	Function	General Name
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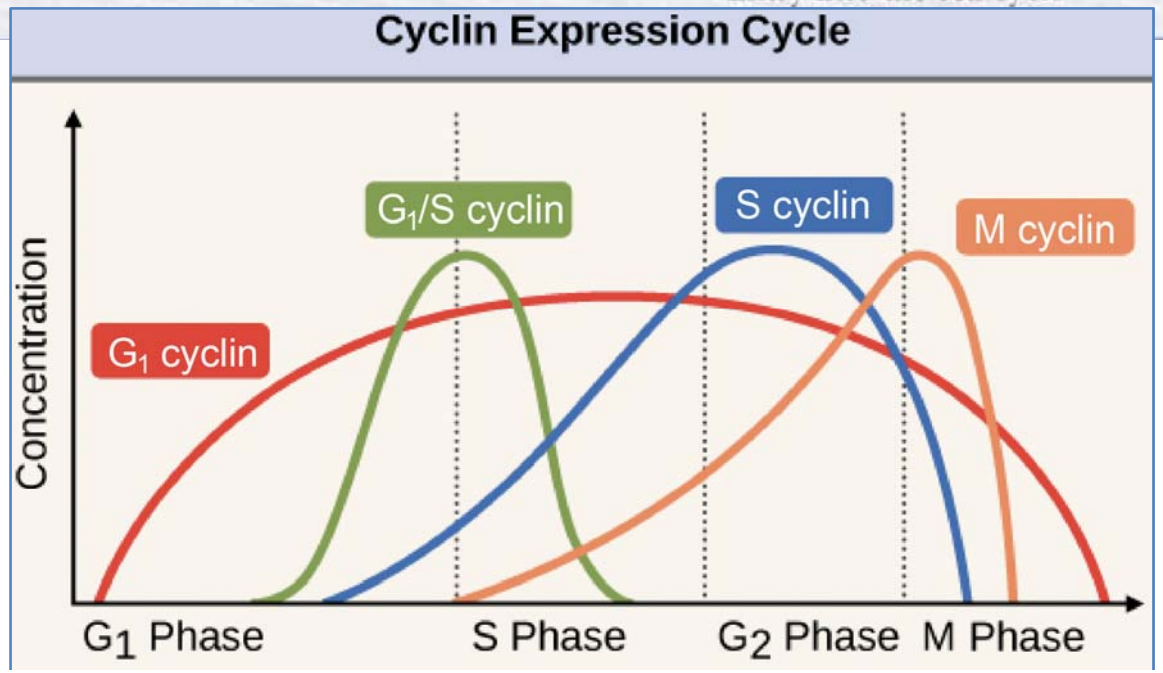


Once activated, mitotic CDKs promote entry into mitosis by phosphorylating and activating hundreds of proteins to promote chromosome segregation and other aspects of mitosis.

Their inactivation during anaphase prompts cells to exit mitosis, which involves the disassembly of the mitotic spindle, chromosome decondensation, the re-formation of the nuclear envelope, and eventually cytokinesis.

Regulation of CDK Activity

Multiple mechanisms ensure that CDKs are active in the right stage of the cell cycle.

CDK activity is regulated by multiple mechanisms:

1. Regualating the cyclin levels
2. Action of CDK-activating kinase (CAK)
3. Inhibitory phosphorylations on CDK
4. Action of CDK Inhibitors

1. Regulation of Cyclin Levels

The timely activation of CDKs depends, in part, on the presence of the appropriate cyclins in the cell cycle stage where they are needed.

Cells utilize multiple mechanisms to restrict cyclins to the appropriate cell cycle stage and to keep them at the right concentration.

Regulation of Cyclin- Mechanisms:

i) Transcriptional control of Cyclin genes

ii) Degradation of cyclins

i) Transcriptional control of the cyclin subunits is one mechanism that ensures proper temporal expression of the cyclins.

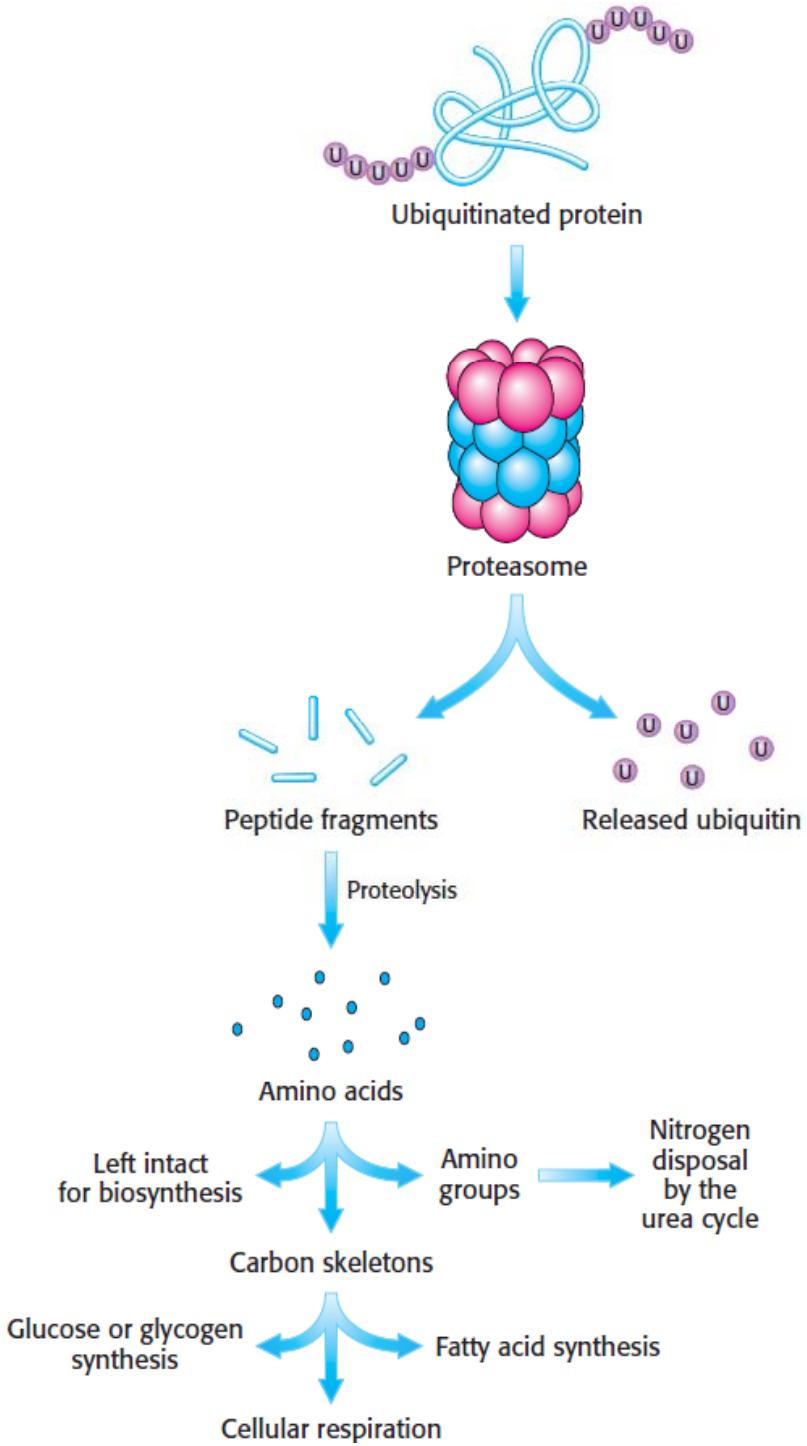
ii) Degradation of cyclins

The most important regulatory control that restricts cyclins to the appropriate cell cycle stage is ubiquitin-mediated protein degradation.

Cyclins are degraded through the action of two different ubiquitin-proteins:

a)SCF (Skp1, Cullin & f-box proteins) and

b)APC/C (anaphase-promoting complex or cydosome).


Ubiquitinated protein
Proteasome
Peptide fragments
Released ubiquitin
Proteolysis
Amino acids
Left intact for biosynthesis
Amino groups
Nitrogen disposal by the urea cycle
Carbon skeletons
Glucose or glycogen synthesis
Fatty acid synthesis
Cellular respiration

SCF controls the G1-S phase transition by degrading G1 cyclins (CyclinD)

The APC/C degrades S phase and mitotic cyclins, thereby promoting the exit from mitosis.

Regulating the levels of cyclins is not the only mechanism that controls CDK activity.

Activating and inhibitory phosphorylation events on the CDK subunit and presence of inhibitors are essential to the control cyclin-CDK activity.

2. CDK-activating kinase (CAK)

Phosphorylation of a threonine residue is required for CDK activity.

This phosphorylation is mediated by the CDK-activating kinase (CAK).

CAK activity is constant throughout the cell cycle and phosphorylates the CDK as soon as a cyclin-CDK complex is formed.

3. Inhibitory phosphorylations on CDK

Inhibitory phosphorylations on CDK play a critical role in controlling CDK activity.

A kinase called "Weel" brings about this inhibitory phosphorylation.

4. CDK Inhibitors (CKIs)

CDK inhibitors control Cyclin-CDK activity

family of proteins known as CDK inhibitors or CKIs, that directly bind to the cyclin-CDK complex and inhibit its activity.

These proteins play an especially important role in the regulation of the G1-S phase transition (entry into the cell cycle).

the genes encoding these CKIs are often found mutated in human cancers.

CKIs involved in regulating S phase and mitotic CDKs are all essential to prevent premature activation of S phase and M phase CDKs.

Inhibitors of G1 CDKs play an essential role in mediating a G1 arrest in response to proliferation inhibitory signals.

A class of CKIs called INK4s interact only with the G1 CDKs.

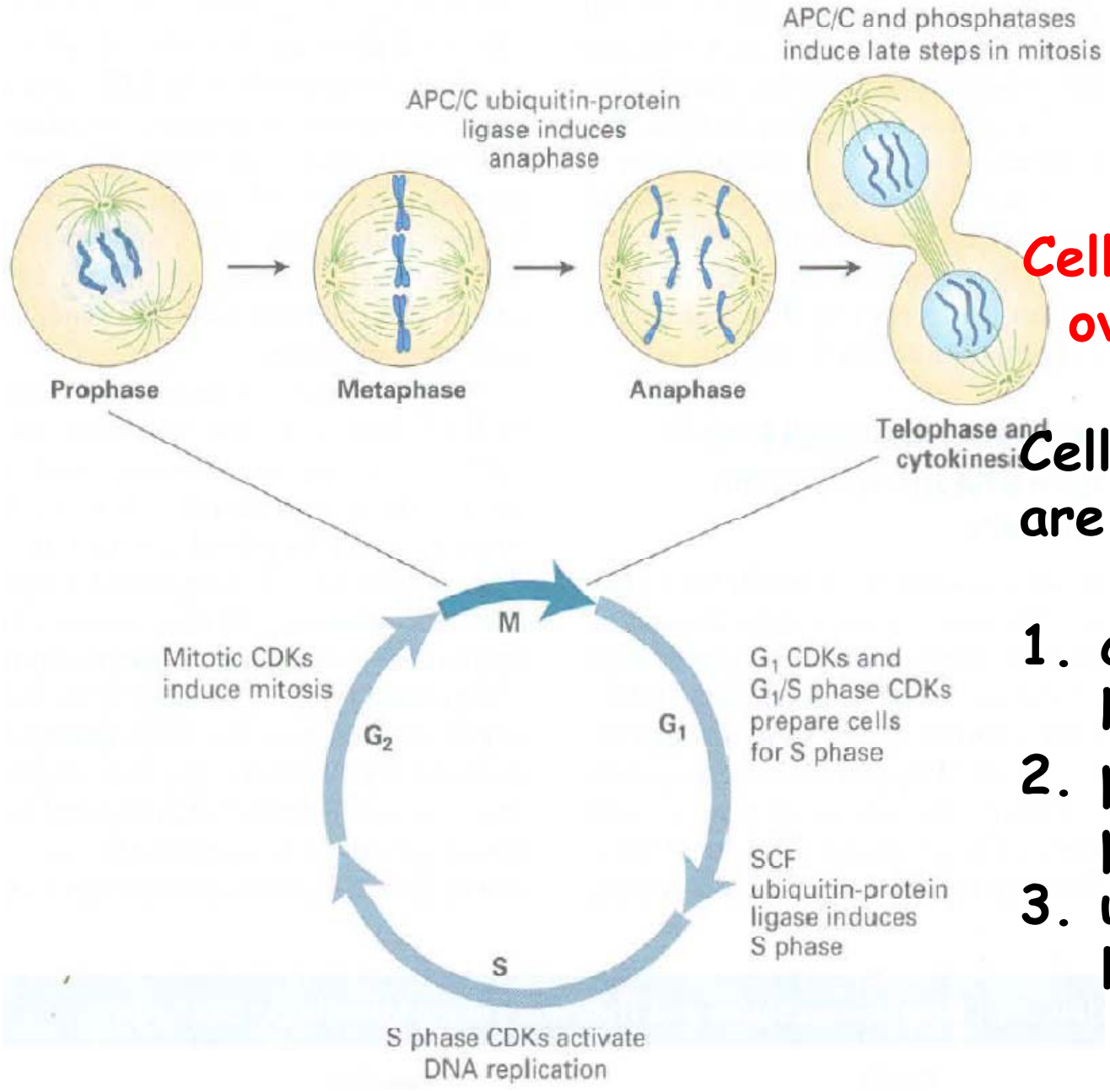
Binding of INK4s to CDK4 and CDK6 blocks their interaction with cyclin D and hence their protein kinase activity.

A second class of CKIs found in metazoan cells consists of three proteins; p21, p27 and p57.

p53 is a TF, which stimulates the expression of p21, p27 and p57 genes.

The p21 protein inhibits cyclin/CDK protein complexes that are needed to progress from the G1 phase of the cell cycle to the S phase

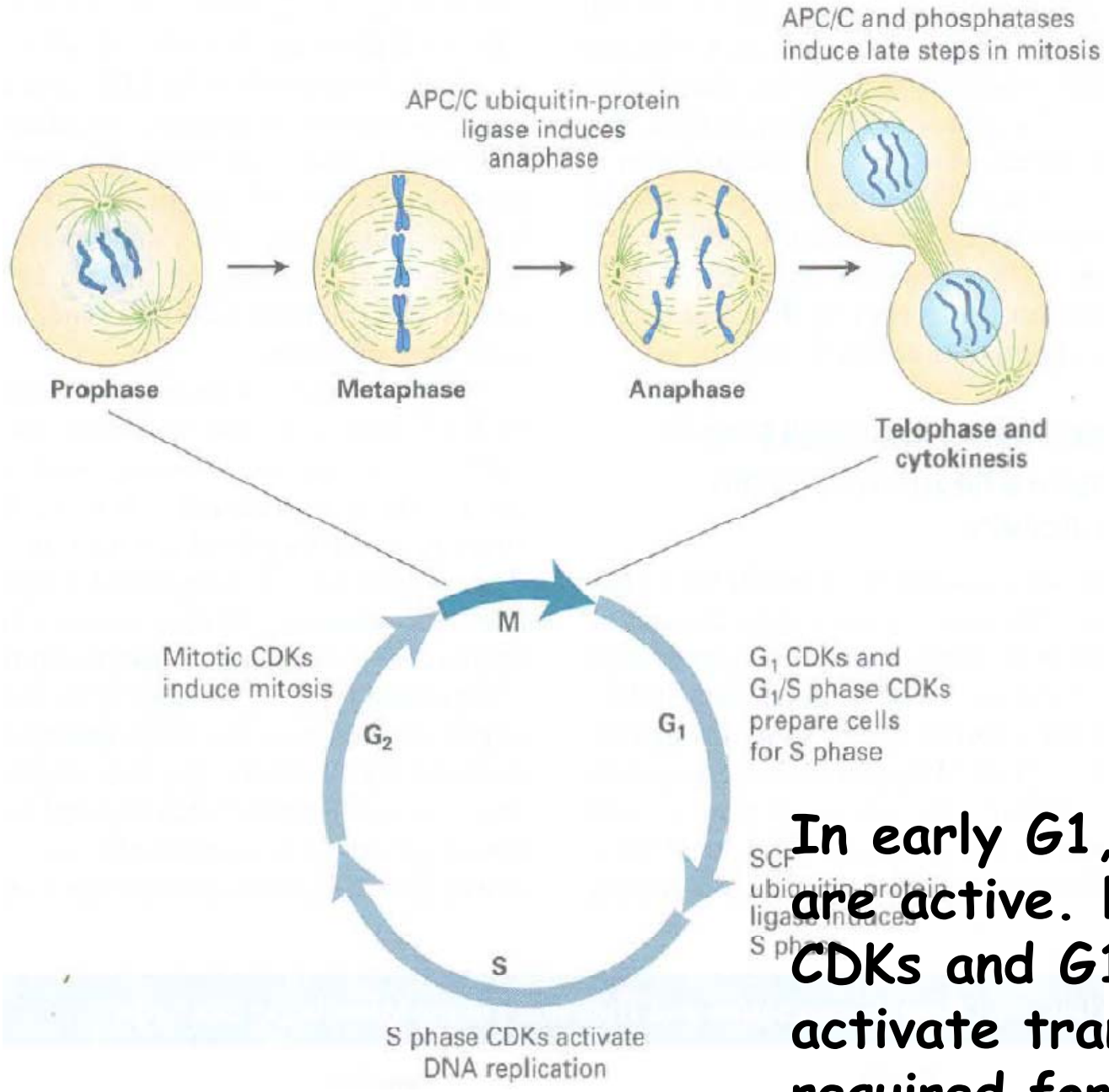
CKIs regulating G1 CDKs play a critical role in preventing tumor formation.



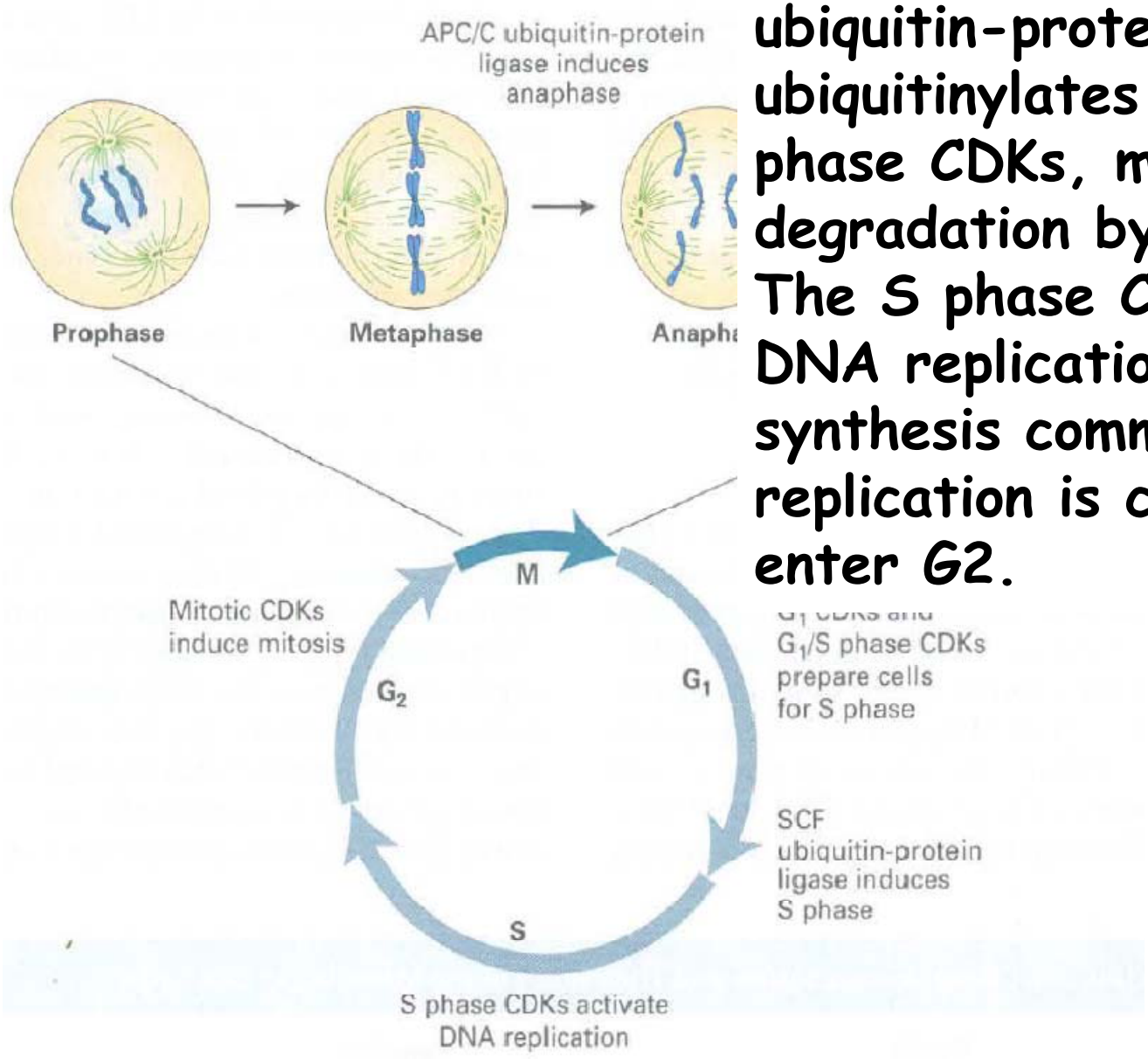
Cell cycle Regulation – over all mechanism

Cell cycle transitions are regulated by

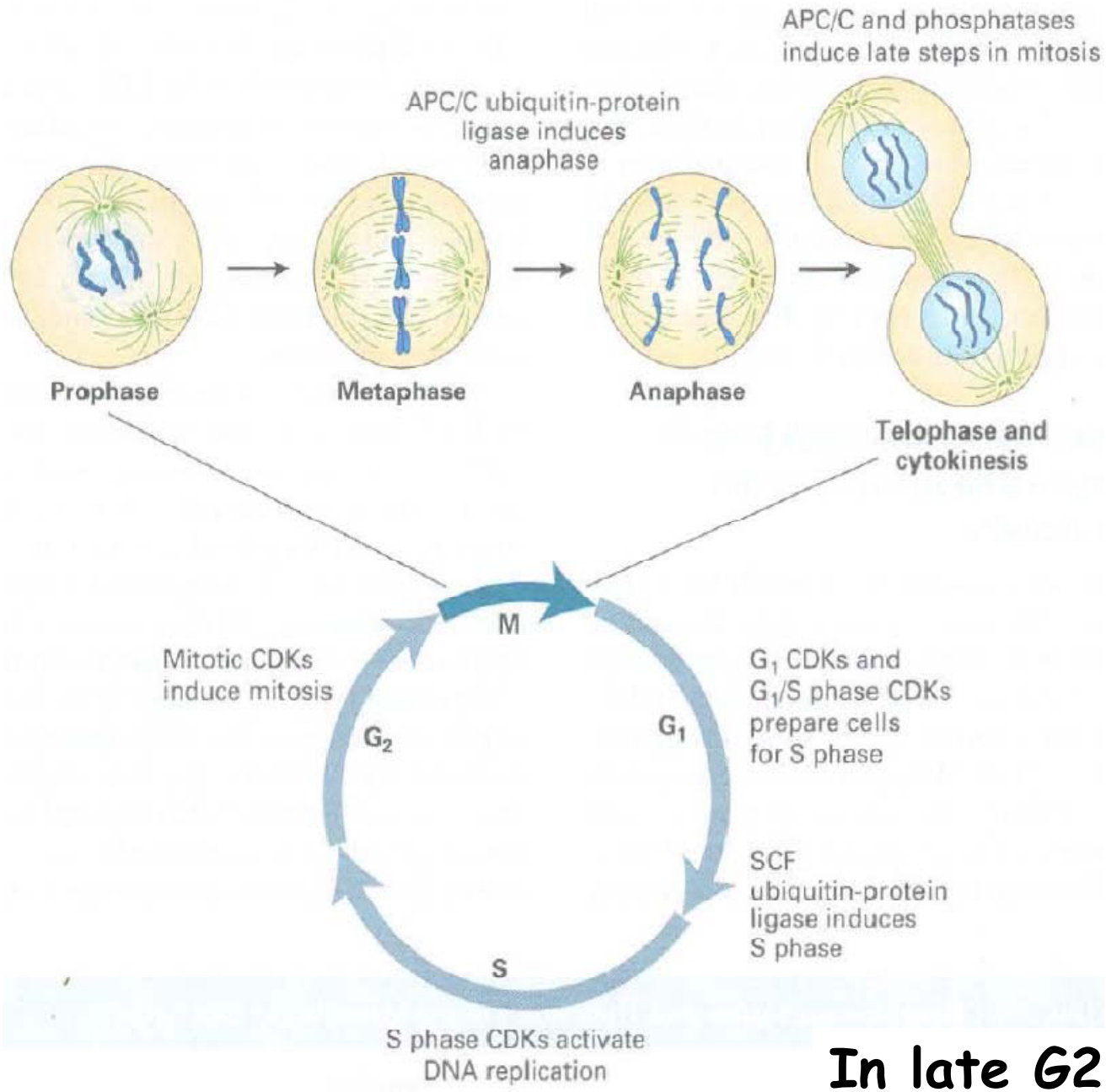
1. **cyclin-CDK protein kinases,**
2. **protein phosphatases and**
3. **ubiquitin-protein ligases**



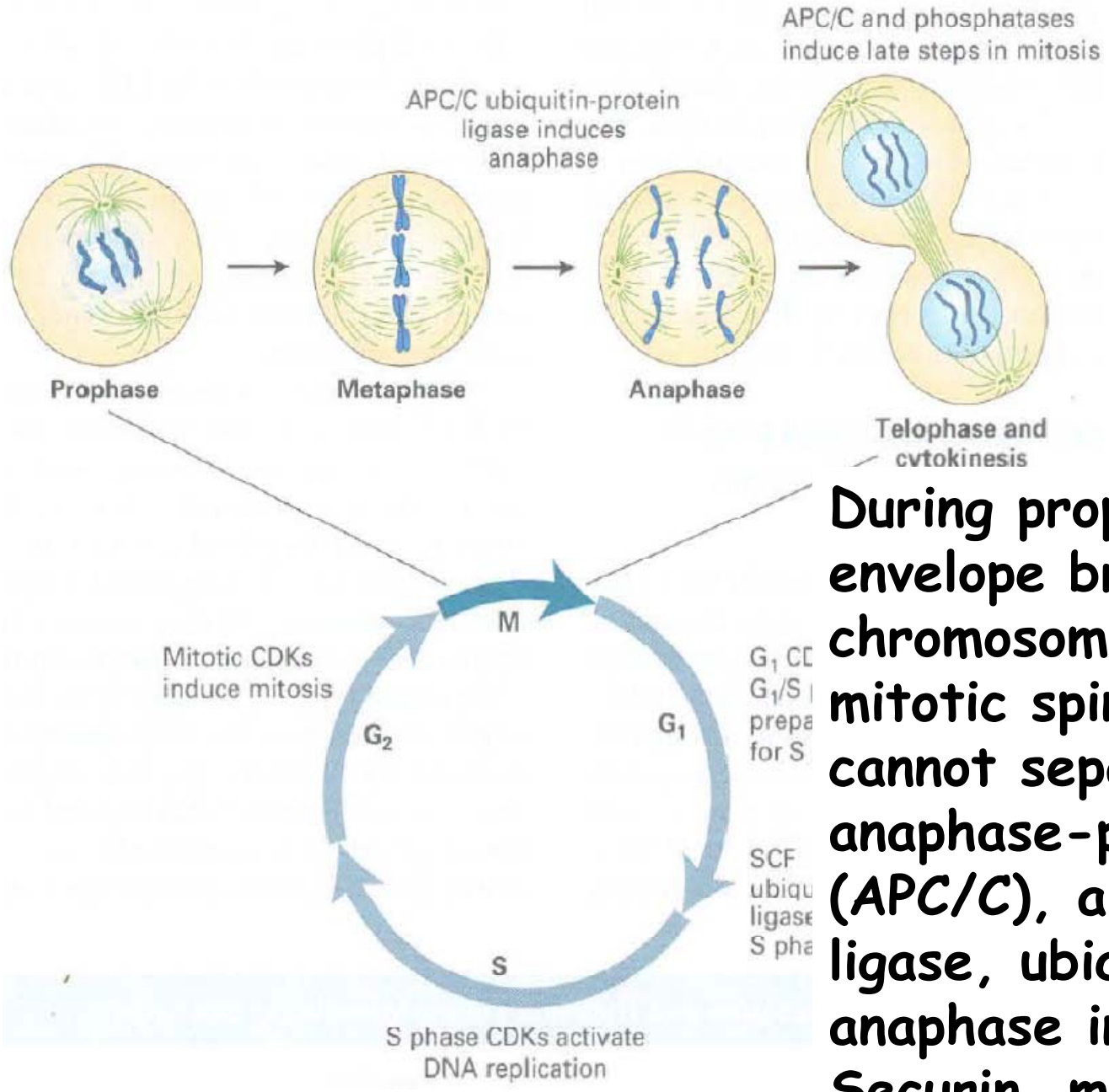
In early G1, no cyclin-CDKs are active. In mid-G1, G1 CDKs and G1/S phase CDKs activate transcription of genes required for DNA replication.



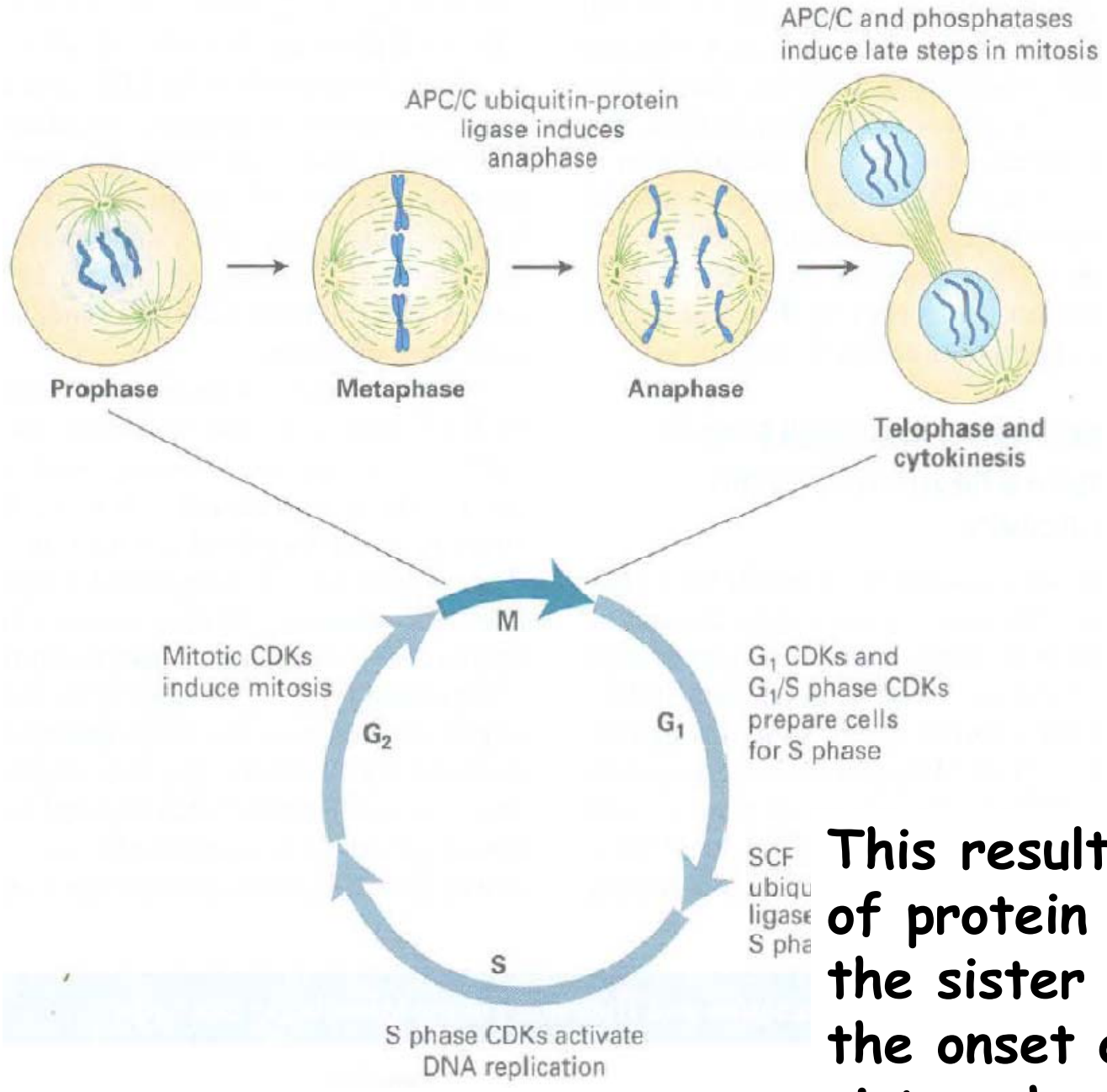
S phase is initiated by the SCF ubiquitin-protein ligase that ubiquitinylates inhibitors of S phase CDKs, marking them for degradation by proteasomes. The S phase CDKs then activate DNA replication and DNA synthesis commences. Once DNA replication is complete, cells enter G2.



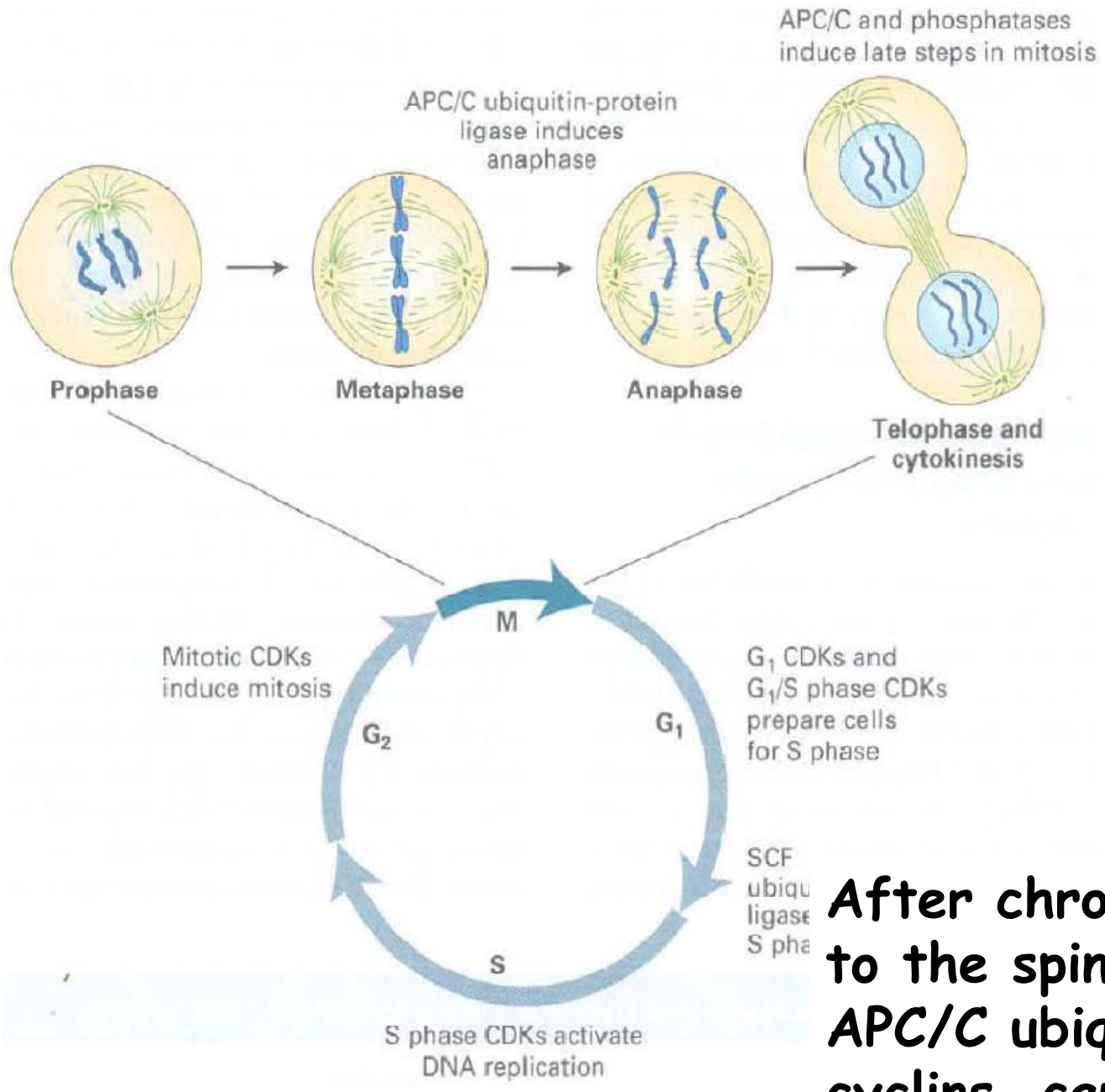
In late G2, mitotic CDKs trigger entry into mitosis.



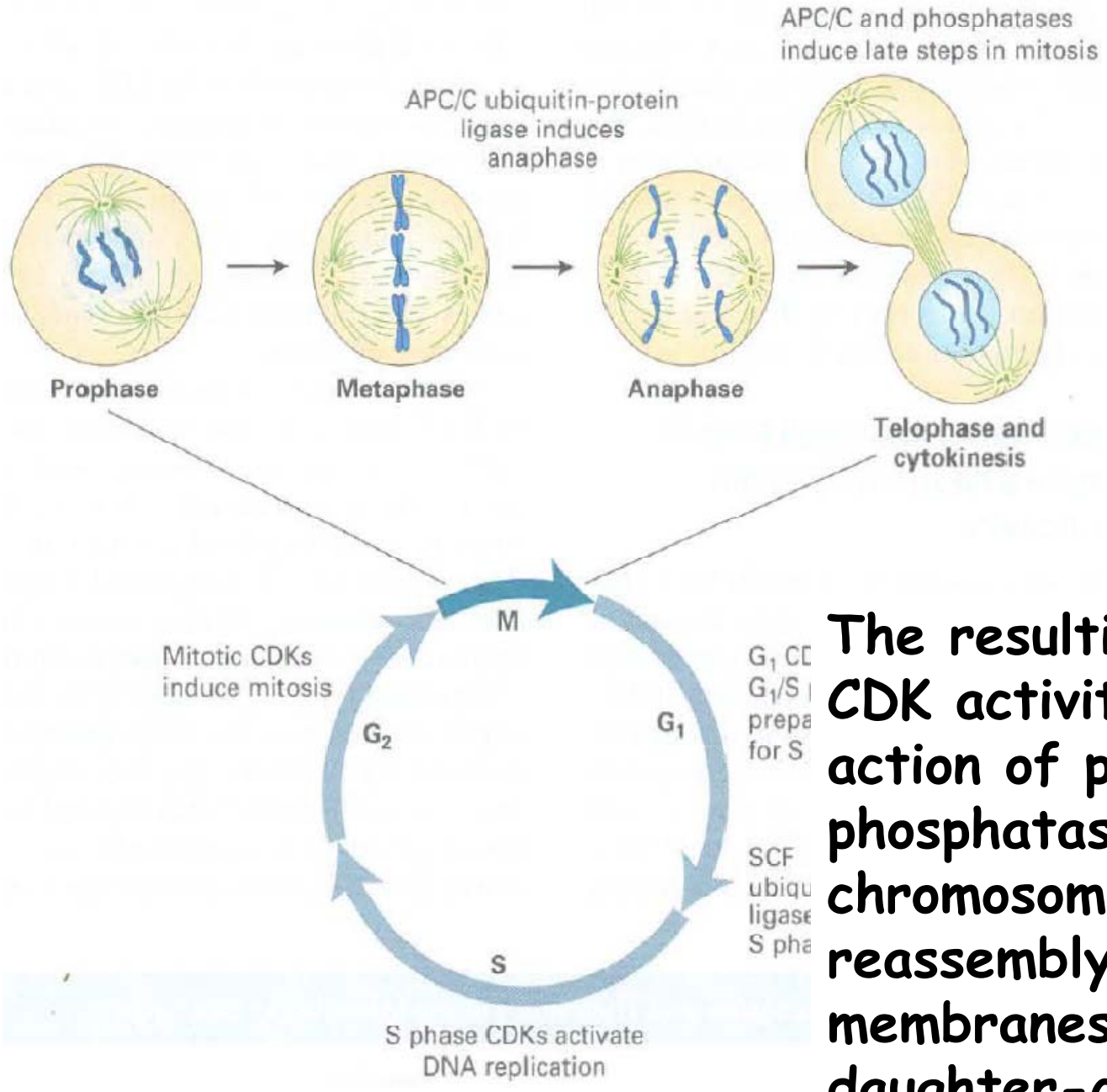
During prophase, the nuclear envelope breaks down and chromosomes align on the mitotic spindle but they cannot separate until the anaphase-promoting complex (APC/C), a ubiquitinprotein ligase, ubiquitinylates the anaphase inhibitor protein Securin, marking it for degradation by proteasomes.



This results in degradation of protein complexes linking the sister chromatids and the onset of anaphase as sister chromatids separate.



After chromosome movement to the spindle poles, the APC/C ubiquitinylates mitotic cyclins, causing their degradation by proteasomes.



The resulting drop in mitotic CDK activity, along with the action of protein phosphatases, results in chromosome decondensation, reassembly of nuclear membranes around the daughter-cell nuclei, and cytokinesis.