Cell cycle checkpoints, Apoptosis and cancer

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CELL CYCLE

- Includes 4 coordinated processes-
  - Cell growth
  - DNA replication
  - Disrtribution of chromosomes
  - Cytokinesis

In bacteria, cell growth and DNA replication takes place throughout the cell cycle.

In eukaryotes, it consists of four phases

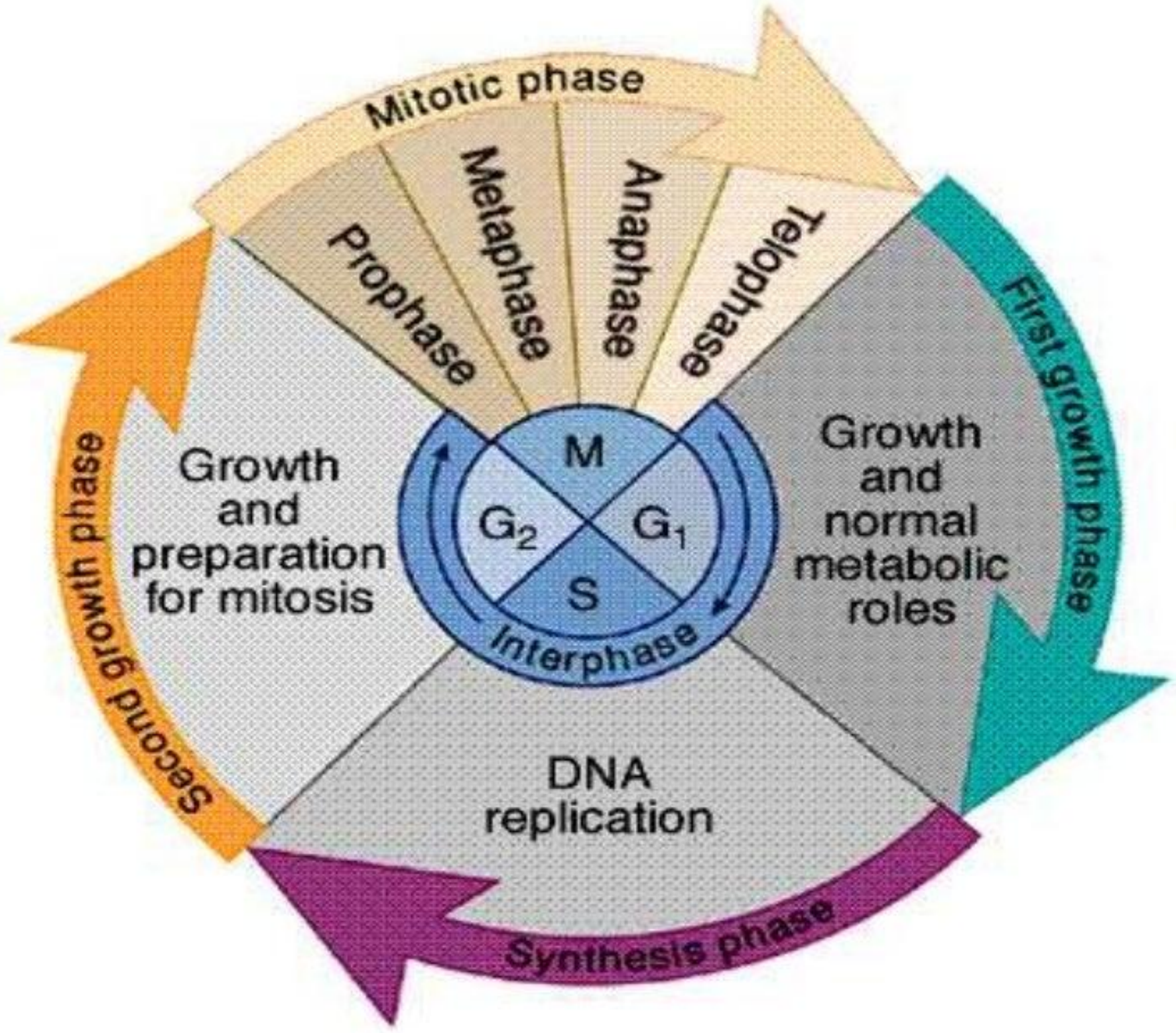
G1 phase – Gap 1

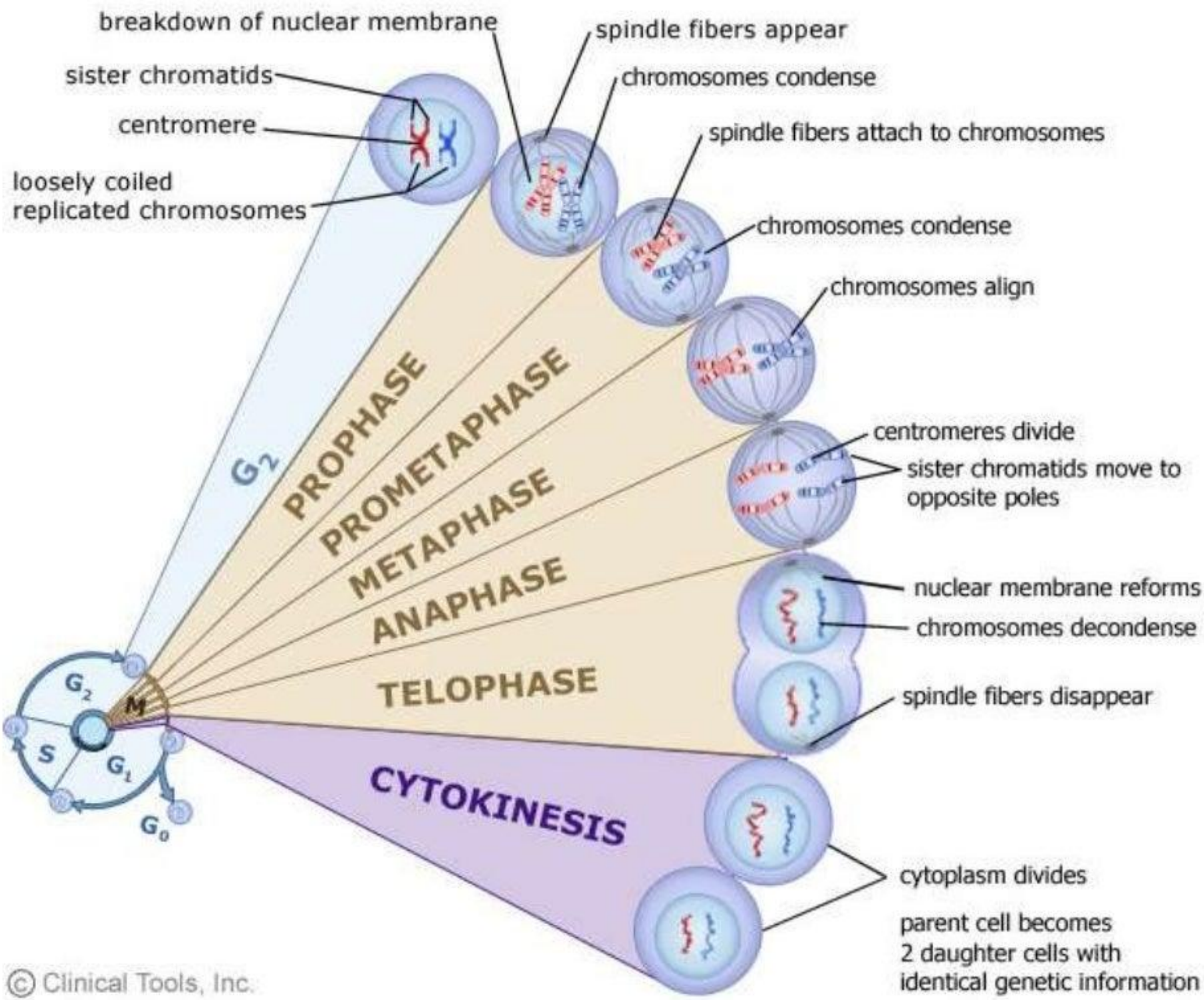
Synthesis phase- S phase

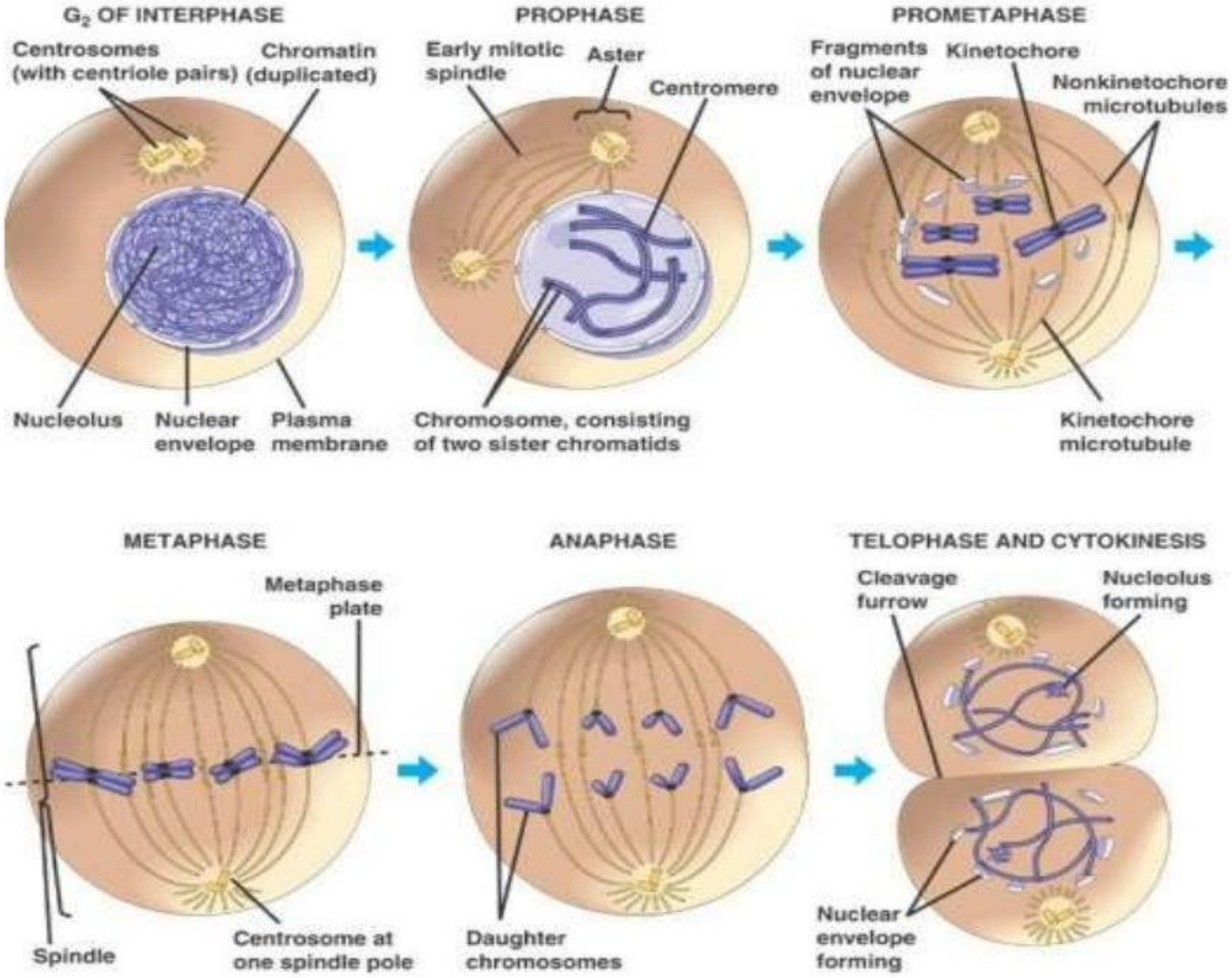
G2 phase- Gap 2

Mitosis phase – M phase

CELL CYCLE


Mitotic phase
Prophase
Metaphase
Anaphase
Telophase
First growth phase
Growth and normal metabolic roles
M
G2
G1
S
Interphase
Growth and preparation for mitosis
Second growth phase
DNA replication
Synthesis phase




G2 OF INTERPHASE
Centrosomes (with centriole pairs)
Chromatin (duplicated)
Nucleolus
Nuclear envelope
Plasma membrane
PROPHASE
Early mitotic spindle
Aster
Centromere
Chromosome, consisting of two sister chromatids
PROMETAPHASE
Fragments of nuclear envelope
Kinetochore
Nonkinetochore microtubules
Kinetochore microtubule
METAPHASE
Metaphase plate
Spindle
Centrosome at one spindle pole
ANAPHASE
Daughter chromosomes
TELOPHASE AND CYTOKINESIS
Cleavage furrow
Nucleolus forming
Nuclear envelope forming

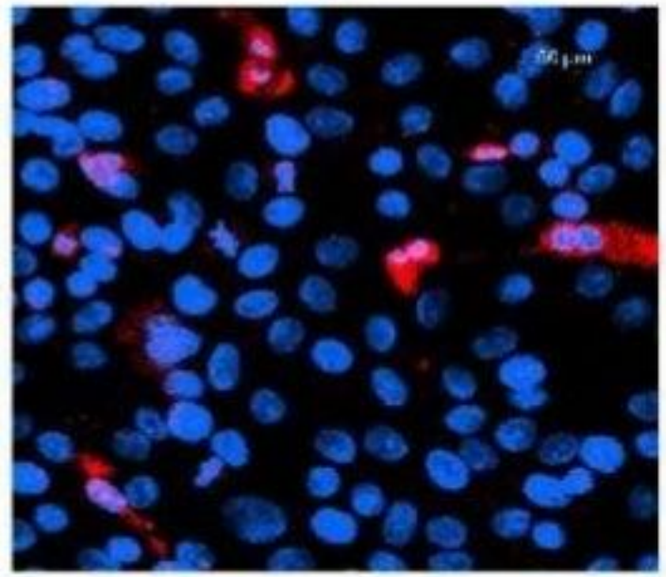
STUDY OF CELL CYCLE PROGRESSION

Different systems

-- yeast: cell cycle mutations

-- frog: big dividing embryos

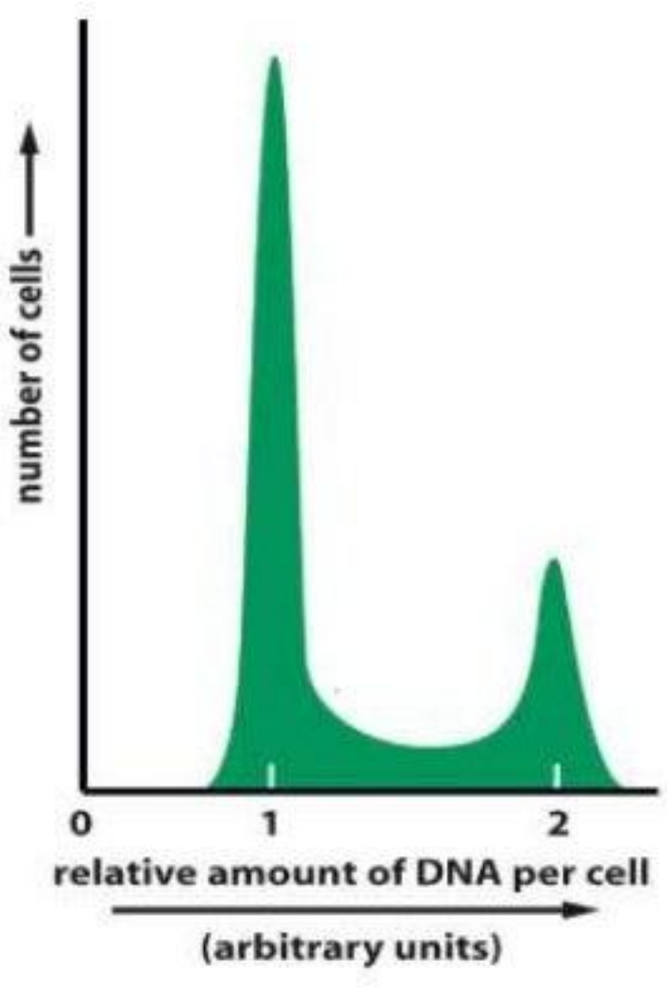
-- sea urchin & clam: many embryos



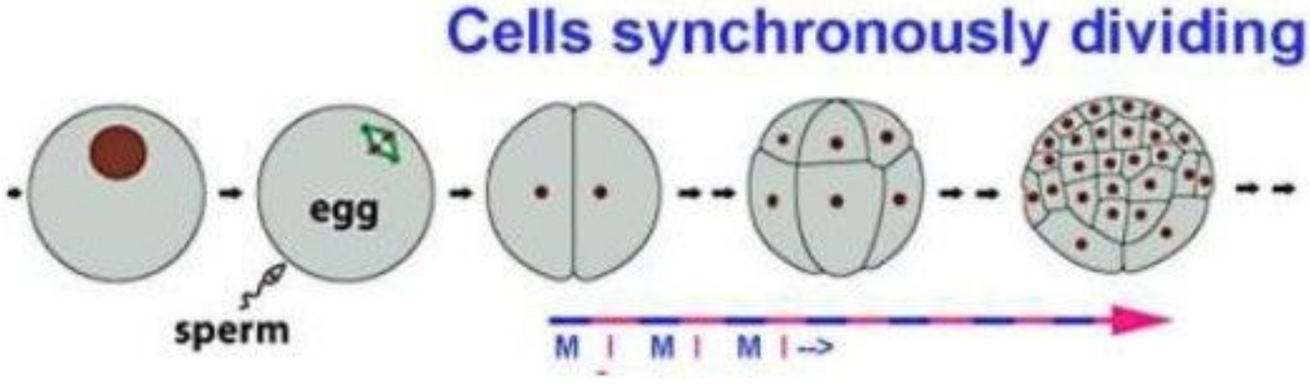
DAPI stained cells

Asynchronously dividing cells

- DNA/nucleus staining
- Flow cytometry

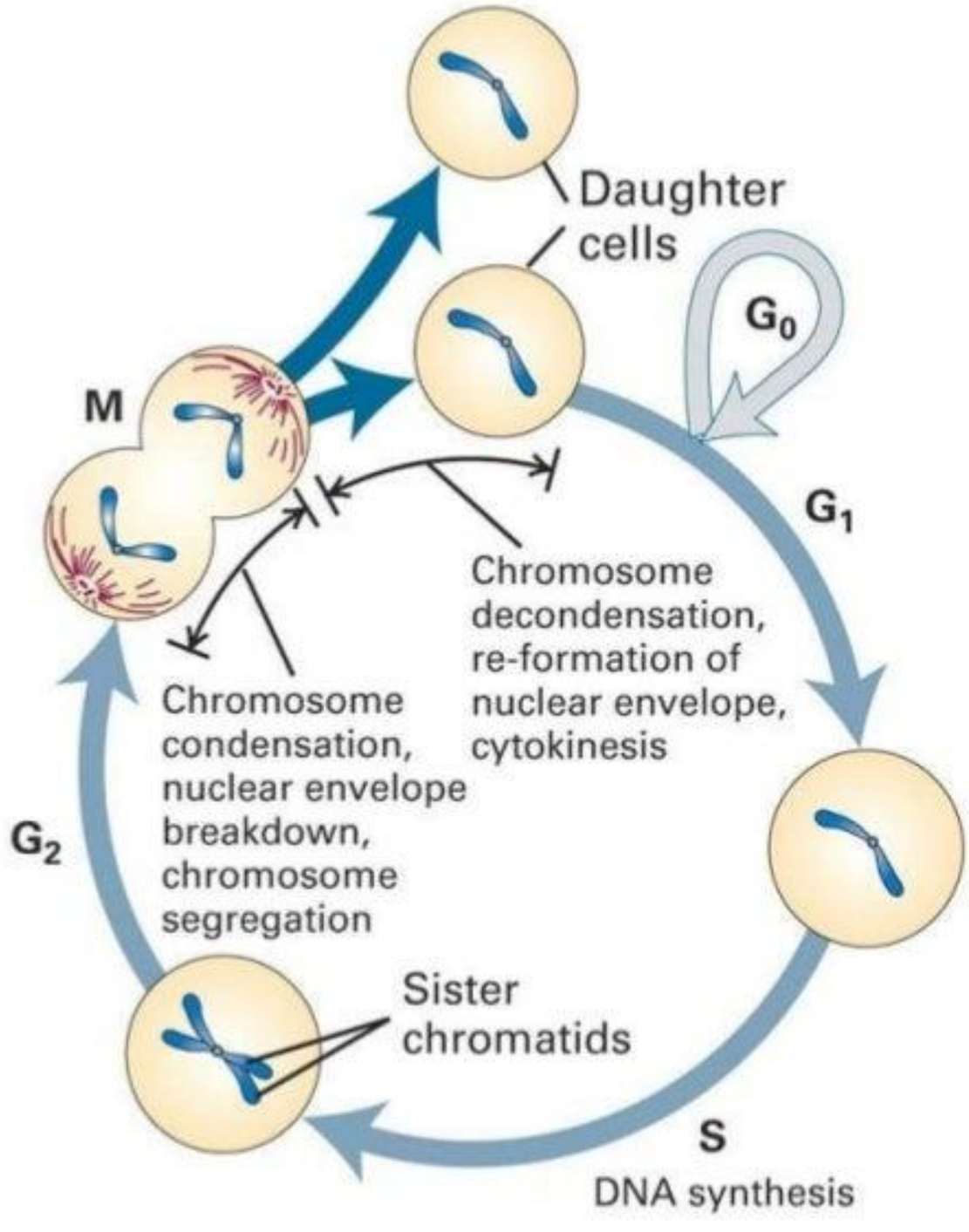


Synchronously dividing cells



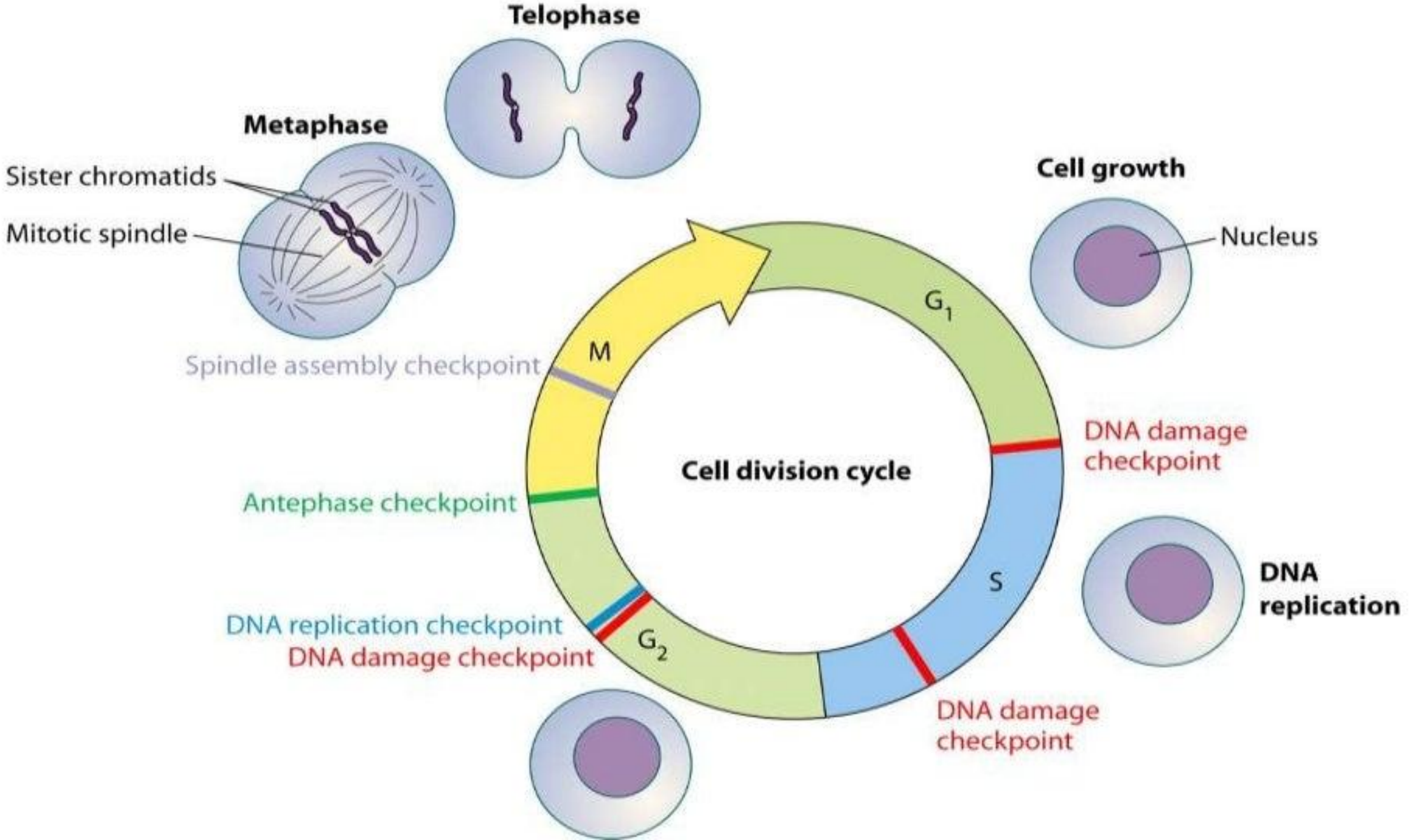
REGULATION OF CELL CYCLE BY EXTERNAL FACTORS

- Major regulatory point in G1 in *Saccharomyces cerevisiae*
- Called START
- Once START is passed cells are commited to enter the S phase.
- It is highly regulated and controlled by extracelluar signals.
  - **Nutrients**
  - **Mating factors**
  - **Cell size**
- If signals are absent the cells are arrested at G1.
- This quiescent stage is called G_0 in which they are metabolically active but cease growth.
- Eg. Skin fibroblasts, nerve cells

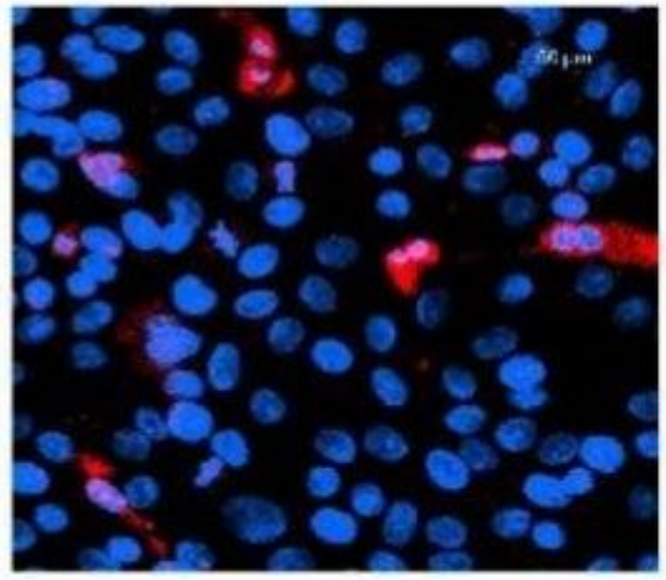


CELL CYCLE CHECKPOINTS

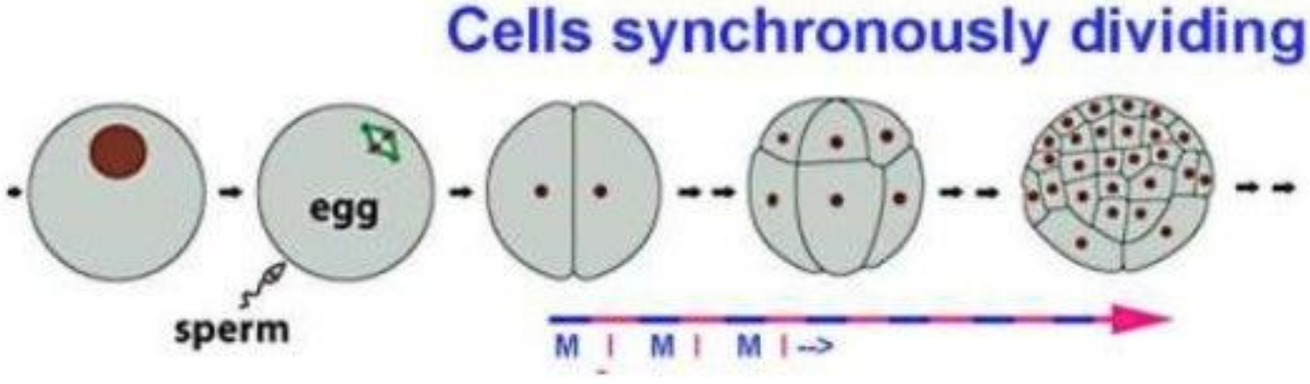
- Prevent entry into next phase of the cell cycle.
- Also called DNA damage checkpoint

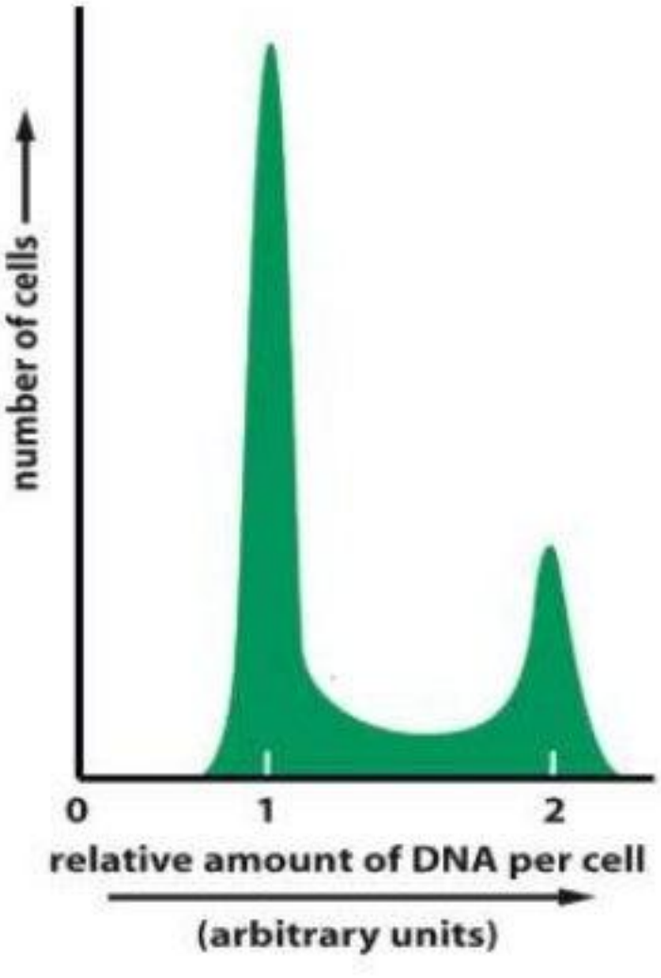


MOLECULES OF CELL CYCLE REGULATION

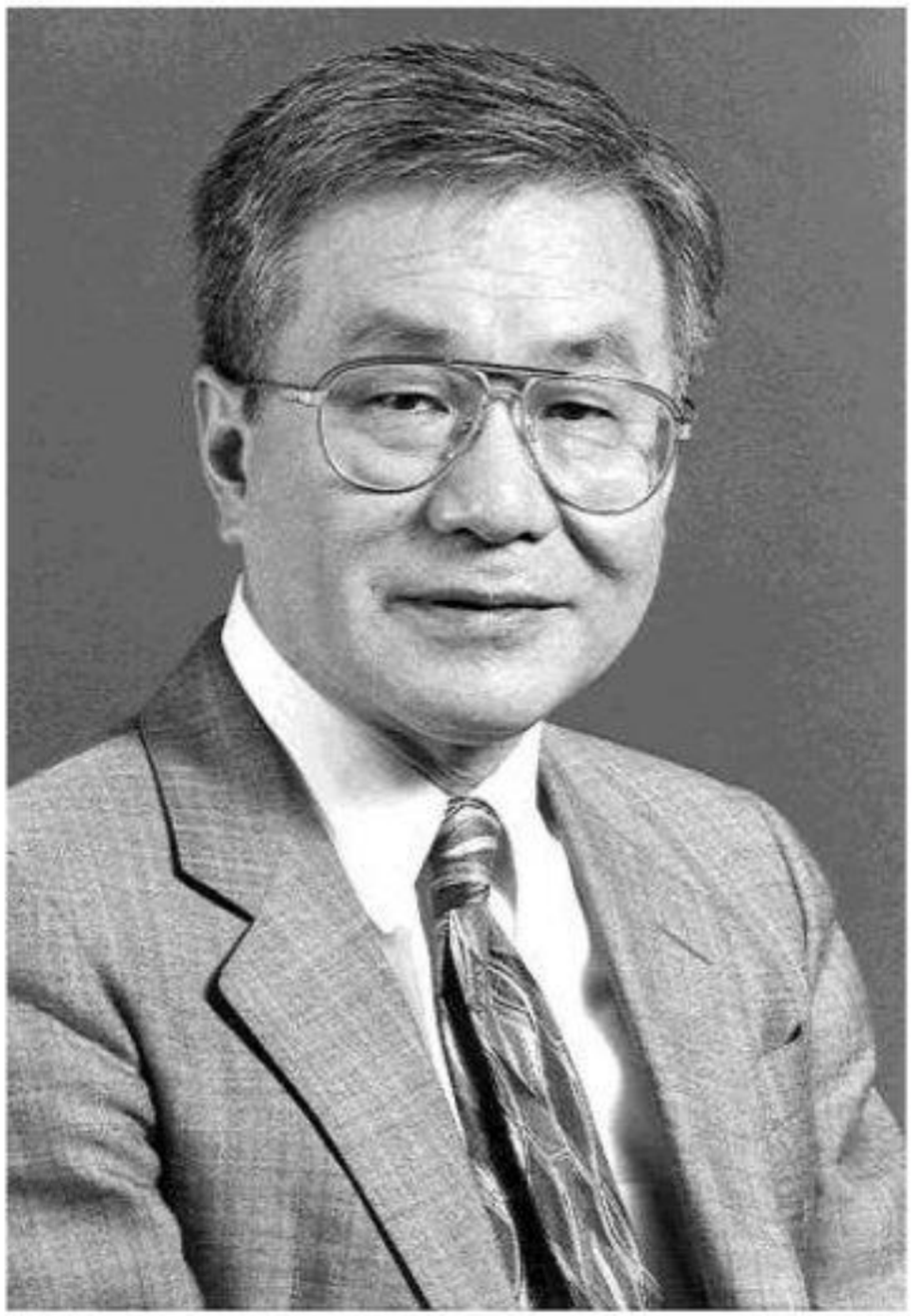


- Three experimental approaches contributed to identification of key molecules responsible for cell cycle regulation
  1. Identification of MPF in frog oocytes
  2. Identification of cdc molecules in *Saccharomyces cerevisiae* mutants
  3. Identification of cyclins in sea urchin embryos

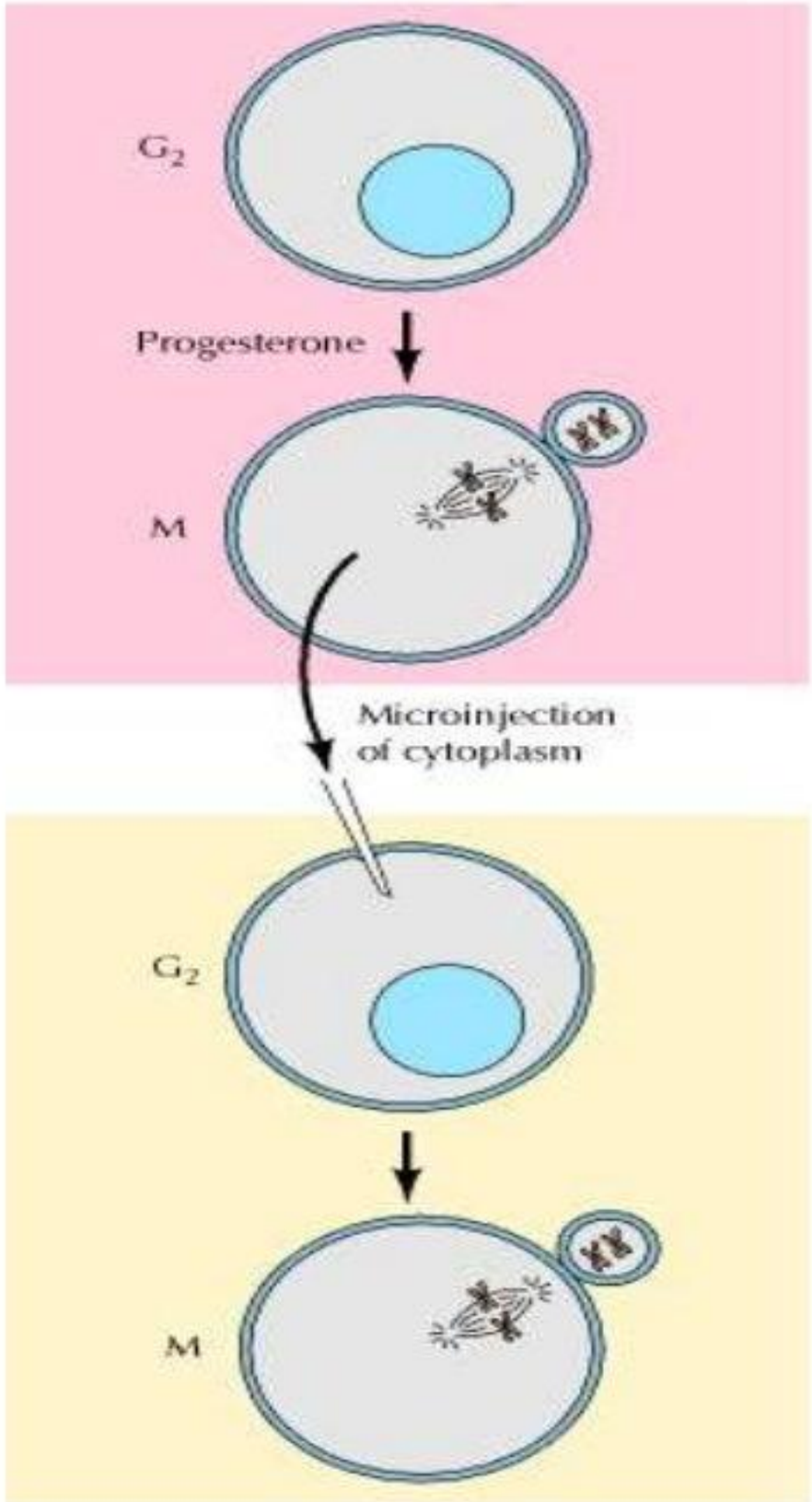


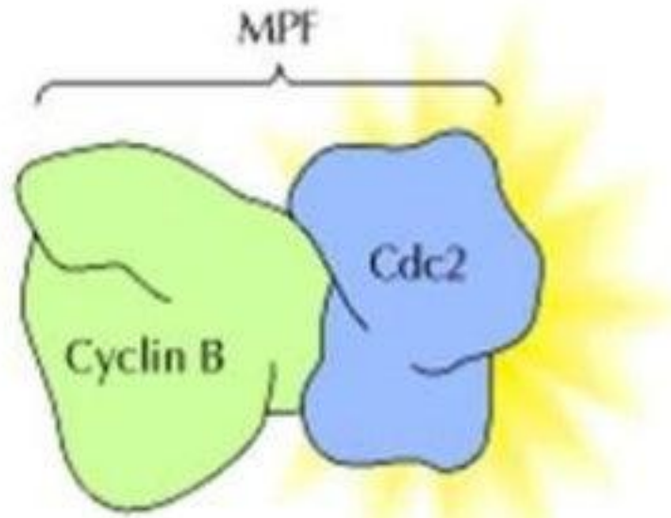


DISCOVERY OF MPF



YOSHIO MASHUI

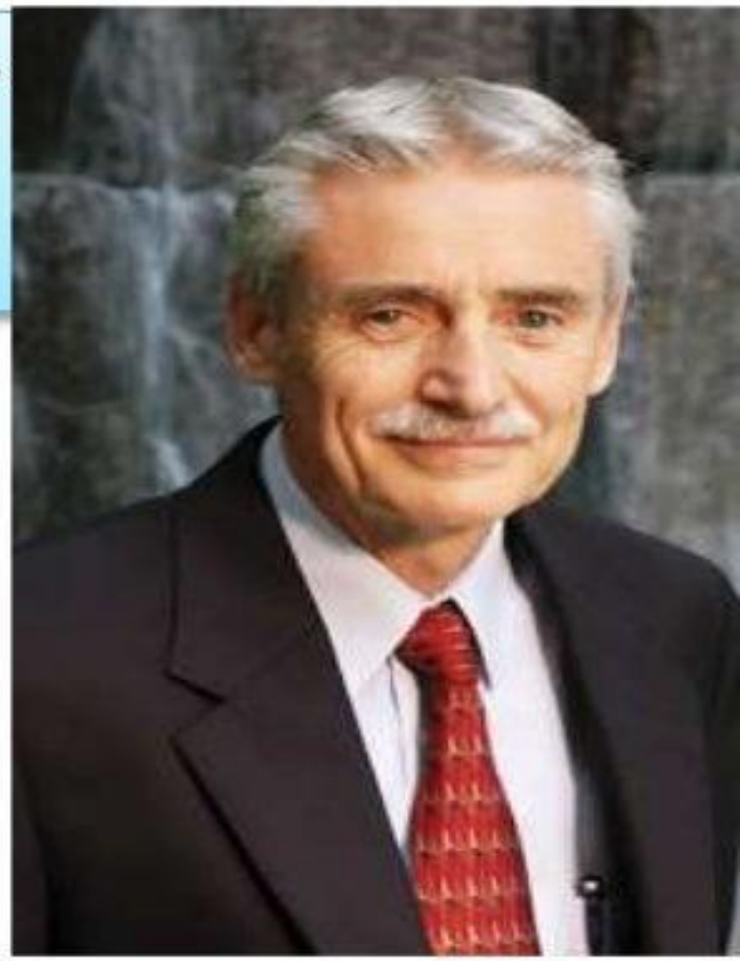




IDENTIFICATION OF CDK

- Studied cdc mutants of *Saccharomyces cerevisiae*
- These required Cdc28 to pass START

PAUL NURSE

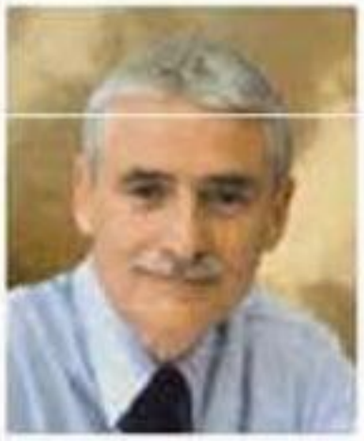


LEE HARTWELL

- Studied cdc mutant of *Schizosaccharomyces pombe*
- Discovred cdc2 which arrest cell cycle at G1 and G2 to M transition

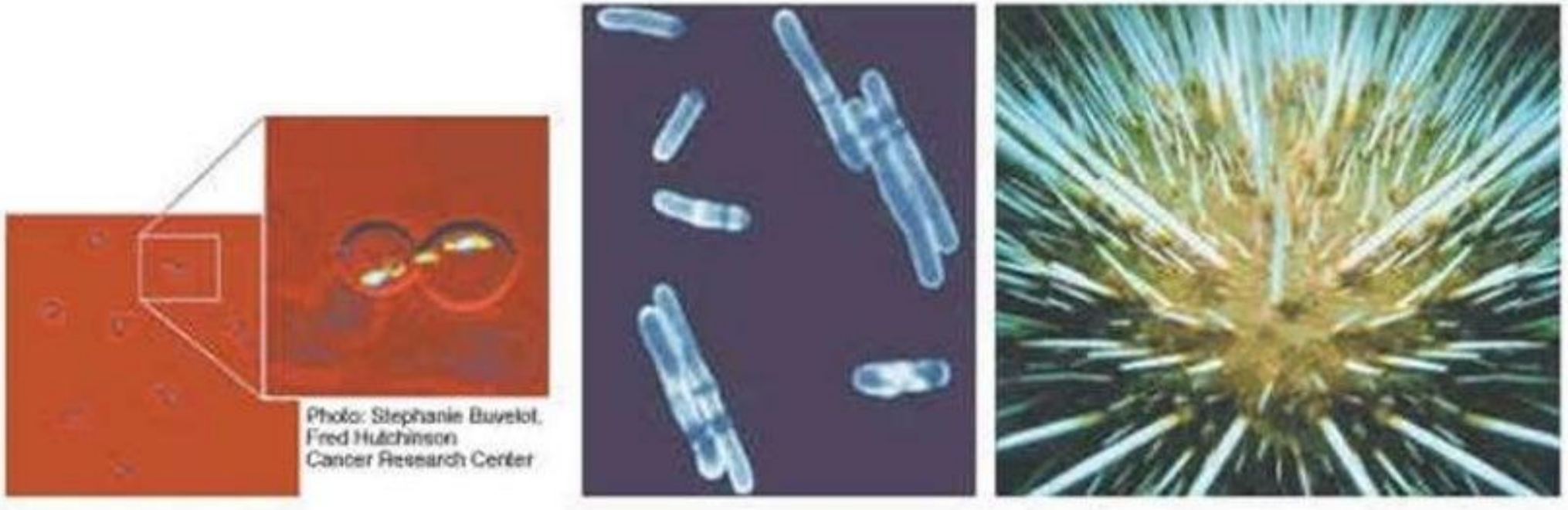
Cdc28 and cdc2 were homologous and coded for a kinase known as **Cdk1**

Leland Hartwell



Leland Hartwell, born 1939. Fred Hutchinson Cancer Research Center, Seattle, WA, USA.

Leland Hartwell used baker's yeast, *Saccharomyces cerevisiae*, as a model system for genetic studies of the cell cycle. In an elegant series of experiments 1970-71, he isolated yeast cells, in which genes controlling the cell cycle were altered (mutated). By this approach, he identified genes specifically involved in cell cycle control, so called CDC-genes (cell division cycle genes). One of these genes, designated *CDC28*, controls the first step in the progression through the G1-phase of the cell cycle (the function "start"). Hartwell also identified the fundamental role of "checkpoints" in cell cycle control. These checkpoints monitor that all steps in the previous phase have been correctly executed and ensure a correct order between the cell cycle phases.



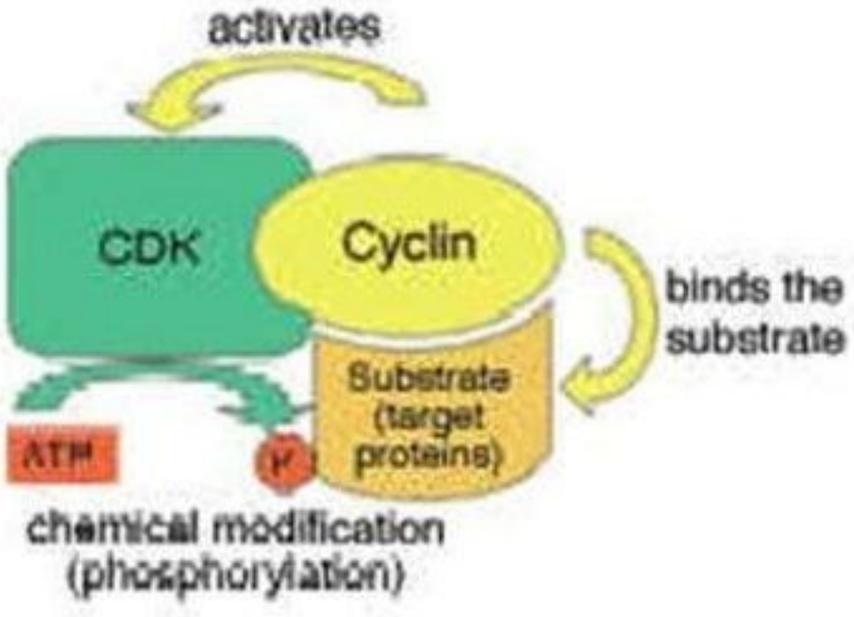
Important model organisms for this year's Laureates. Leland Hartwell used baker's yeast, *Saccharomyces cerevisiae* (left). Paul Nurse used another type of yeast, *Schizosaccharomyces pombe* (middle). Tim Hunt used sea urchin, *Arbacia* (right).

Paul Nurse,
born 1949.
Imperial Cancer
Research Fund,
Lincoln's Inn
Fields, London,
UK.

Paul Nurse identified the key regulator of the cell cycle, the gene *cdc2*, during the years 1976-80. He showed that the product of this gene controls cell division (transition from G2 to M). Nurse discovered the gene *cdc2* in the fission yeast *Schizosaccharomyces pombe*. He later showed that *cdc2* had the same function as the gene *CDC28* in the distantly related baker's yeast.

Thus, *cdc2* has more than one function in the cell cycle, controlling both the transition from G1 to S and G2 to M. In 1987 Paul Nurse isolated the corresponding human gene, later called *CDK1*. These findings showed that the CDK function has been conserved through evolution.

The gene *CDK1* encodes a protein that is a member of a family called cyclin dependent kinases (CDK). These molecules function by linking phosphate groups to other proteins (phosphorylation, figure to the left). Today half a dozen different CDK-molecules have been found in humans.

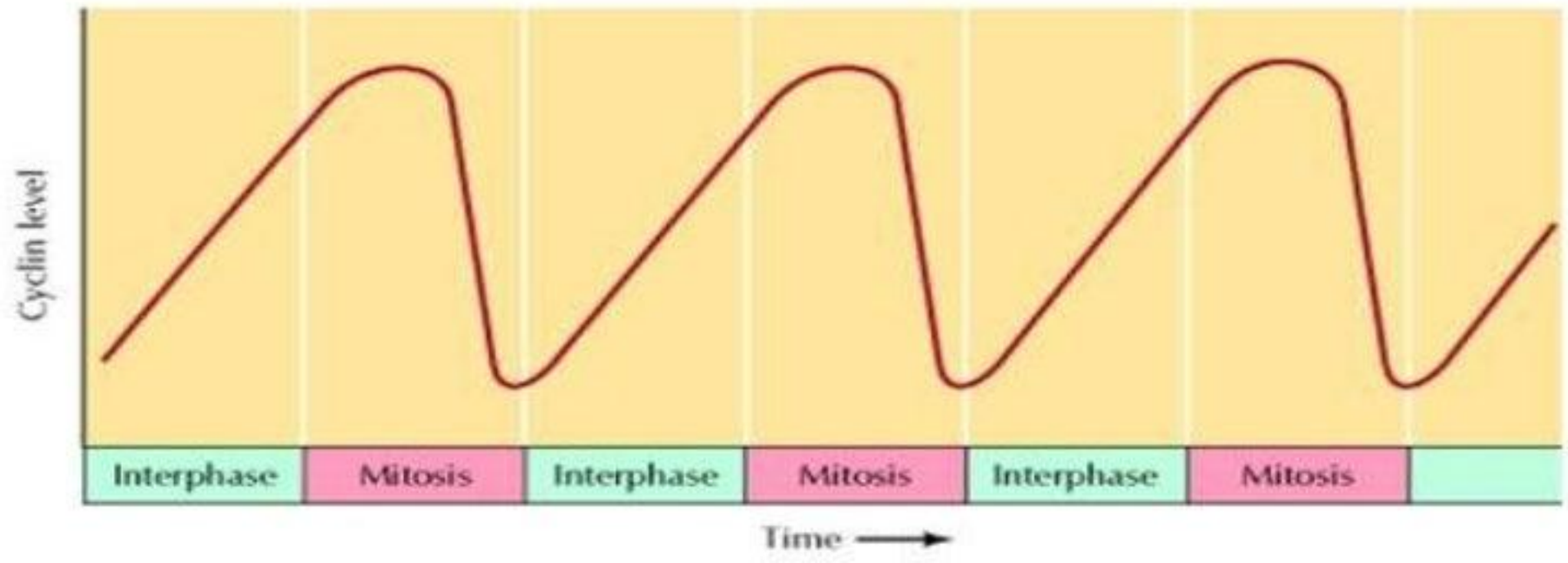


CDK and cyclin together form an enzyme that activates other proteins by chemical modification (phosphorylation). The amount of CDK molecules is constant during the cell cycle, but their activities vary because of the regulatory function of the cyclins. CDK can be compared with an engine and cyclin with a gear box controlling whether the engine will run in the idling state or drive the cell forward in the cell cycle.

IDENTIFICATION OF CYCLINS

- Stuied in sea urchin and calm embryo in 1983
- Accumulation in interphase and degradation in the end of mitosis
- Hunt called these cyclin A and cyclin B
- In 1986, Joan Ruderman showed cyclin A triggers G2 to M transition in frog oocyte

- Accumulation and degradation of cyclins

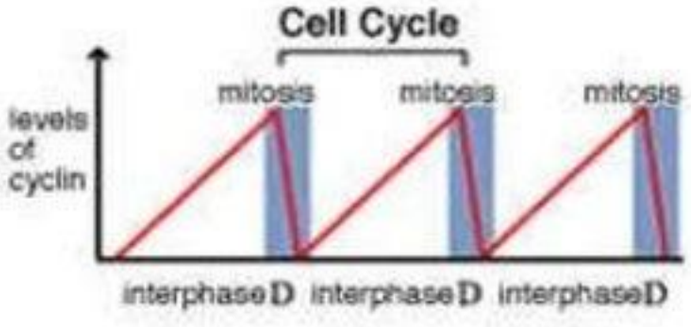


Tim Hunt

Tim Hunt,
born 1943.
Imperial Cancer
Research Fund,
Clare Hall
Laboratories,
South Mimms,
UK.

Tim Hunt discovered cyclins, proteins that bind to the CDK molecules. Cyclins regulate the CDK activity and select the target proteins to be phosphorylated. The proteins were named cyclins because of their cyclic variation in amount during the cell cycle (figure bottom left). Hunt's discovery that cyclins were degraded during mitosis turned out to be another fundamental control mechanism in the cell cycle.

Tim Hunt discovered the first cyclin molecule in 1982, using eggs from sea urchin, *Arbacia*, as a model system. He also found that cyclins, like CDK, were conserved during evolution. Today around ten different cyclins have been found in humans.



Cyclins are proteins formed and degraded during each cell cycle. Periodic protein degradation is an important control mechanism of the cell cycle. (D = cell division.)

The fundamental molecular mechanisms controlling the cell cycle are highly conserved through evolution and operate in the same manner in yeasts, insects, plants, animals and humans.

Photos: Eva Löfman, Carl Löfman

STRUCTURE OF MPF

- Purified in 1988 by James Maller from frog eggs
- MPF is composed of two subunits
  - Cdk1 – catalytic subunit
  - Cyclin B – regulatory subunit

- Cyclin B is synthesized and form complexes with cdk1 during G2.
- Phosphorylation of cdk2 at threonine 161 is required for its activity
- Phosphorylation of tyrosine 15 by wee1, inhibits cdk1 activity and leads to the accumulation of cdk and cyclin B complex.
- Activation is by deposphorylation of threonine 14 and tyrosine 15 by the phosphotase cdc25 for G2 to M transition

Replication completed

Checkpoint proteins

Chk1

Cdc25 Active

Cyclin B

Cdc2

Inactive MPF

Cyclin B

Cdc2

Active MPF

Progression to mitosis

Unreplicated or damaged DNA

Checkpoint proteins

Chk1

Cdc25 Inactive

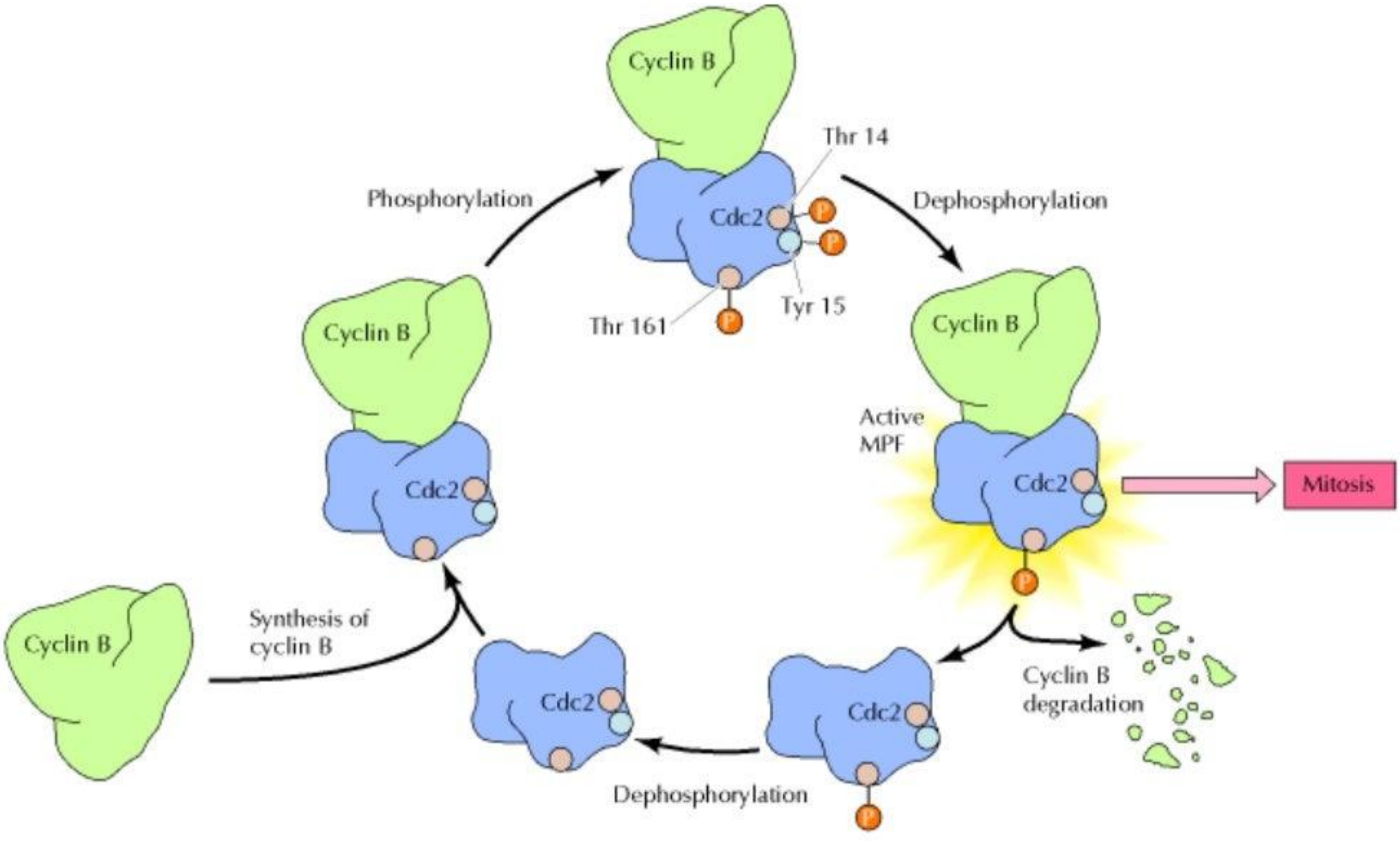
Cyclin B

Cdc2

Inactive MPF

G_2 arrest

MPF Regulation



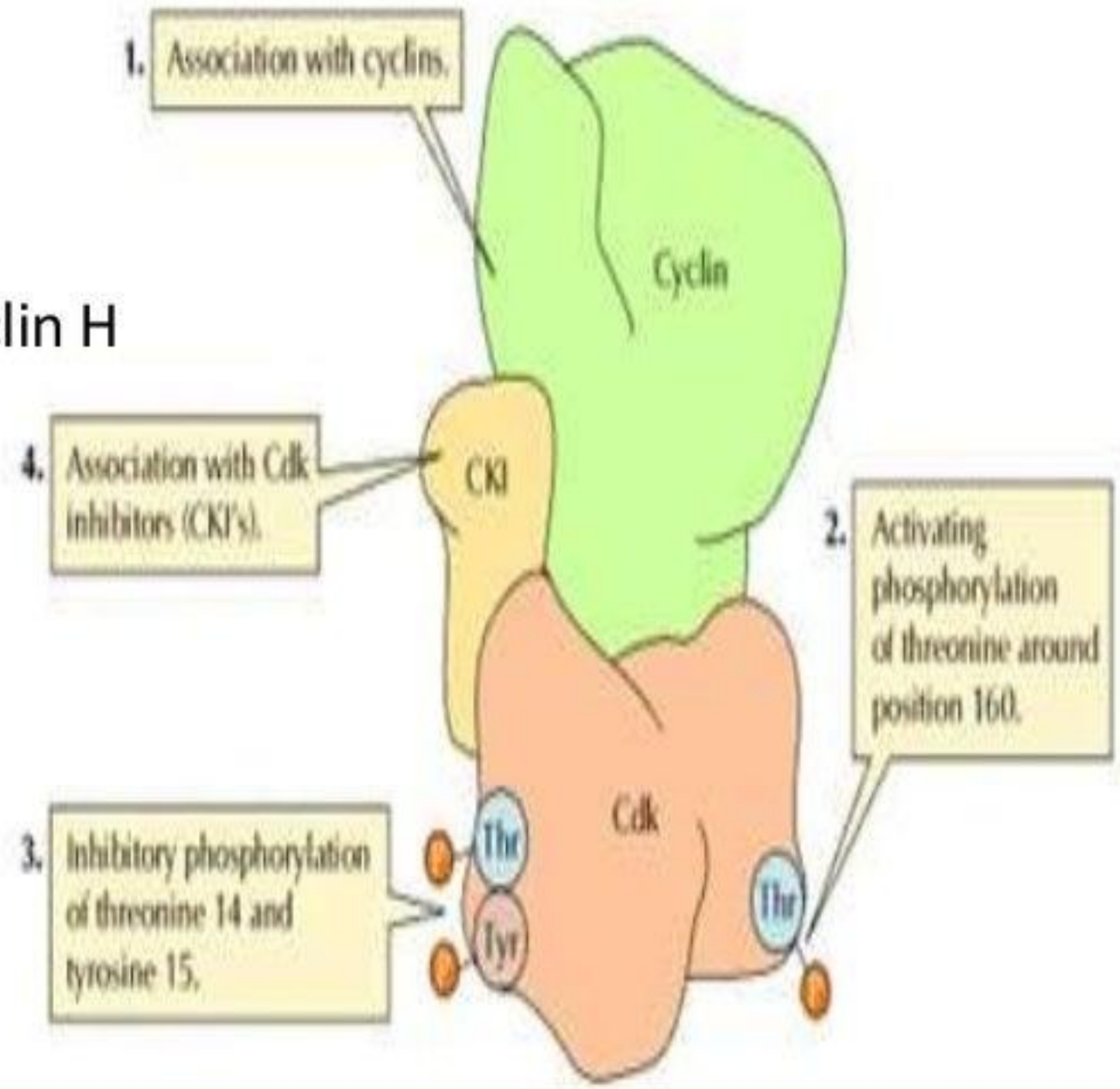
Mechanism of cdk regulation

- Association with cyclins

- Phosphorylation at threonine 160
- by Cak composed of cdk7 and cyclin H

- Inhbitory Phosphorylation at
- threonine 14 and tyrosine 15

- 2 families of cdk inhibitors
  - Ink family
  - cip/kip family



•Ink family **(p15, p16, p18, p19**)	cdk4/cdk6	G1
•Cip/kip family (**p21, p27, p57**)	cdk2/cyclin E	G2
	cdk2/cyclin A	S

Families of cyclins and cyclin dependent kinases

- In eukaryotes
  - **G1 to S** - cdk2, cdk4,cdk6 + cyclin D & E
  - $\mathbf{G_0}$ – cdk4, cdk6 + cyclin D1,D2 & D3
  - **Late G1** - cdk2 + cyclin E1 &E2
  - **Through S** – cdk2 + cyclin A1 & A2
  - **S to G2** – cdk1 + cyclin A1 & A2
  - **G2 to M** – cdk1 + cyclin B1, B2, & B3