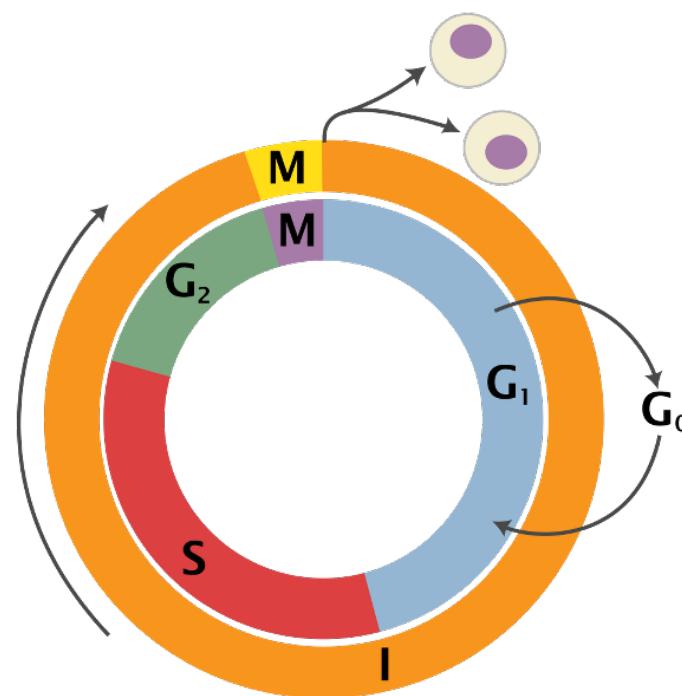


Introduction to Molecular and Cellular Biology

LECTURES 21-22:

Cell cycle I



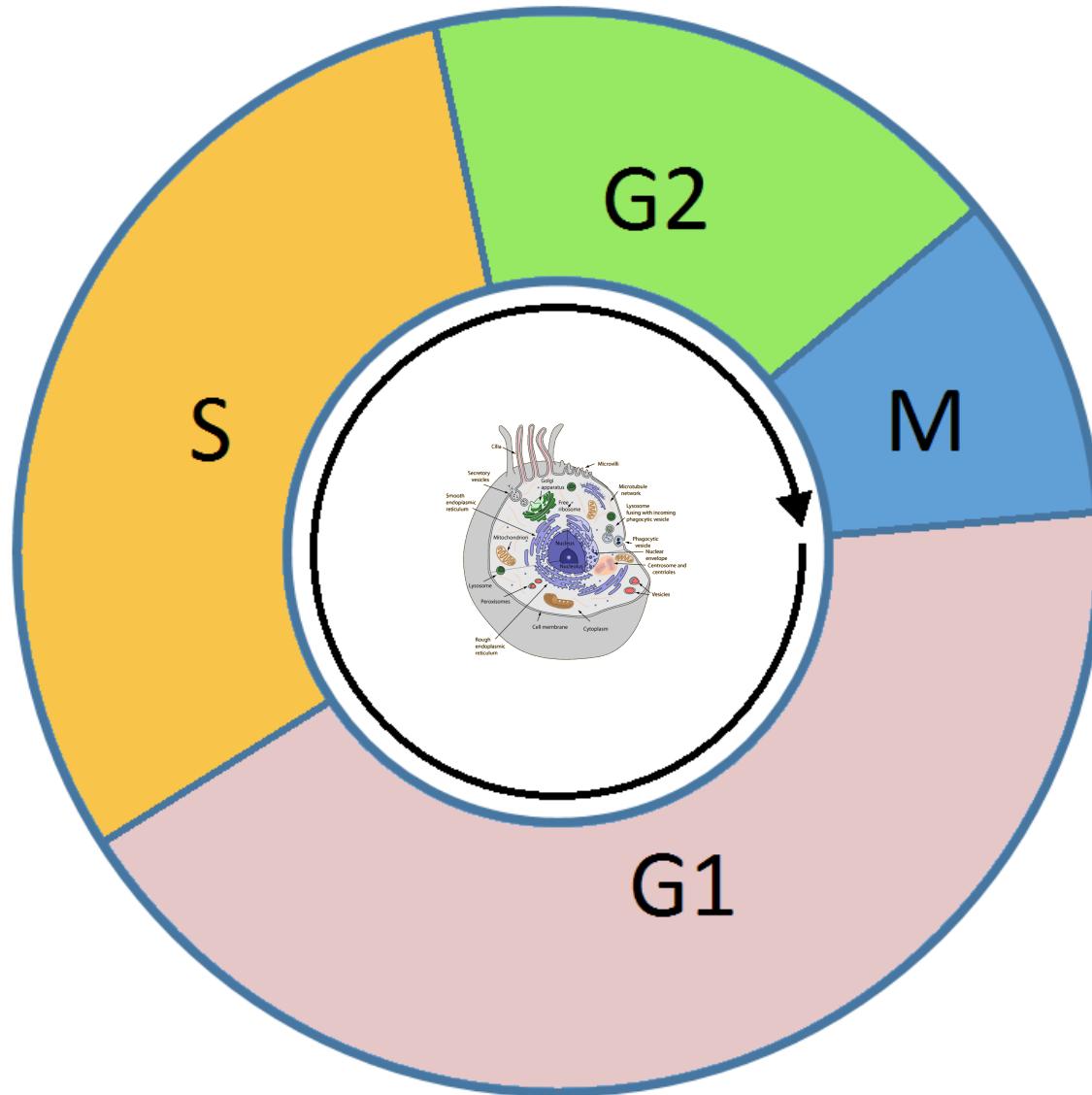
LECTURES 21-22: CELL CYCLE I

- Introduction to the cell cycle: principles and discoveries
- Phases of cell cycle and their control:
 - interphase (S , G1 , G2 phases)
 - M phase (mitosis, cytokinesis)
- Apoptosis



INTRODUCTION:

The series of events taking place in a cell leading to its division and duplication of its DNA to produce two daughter cells



G1 - Growth

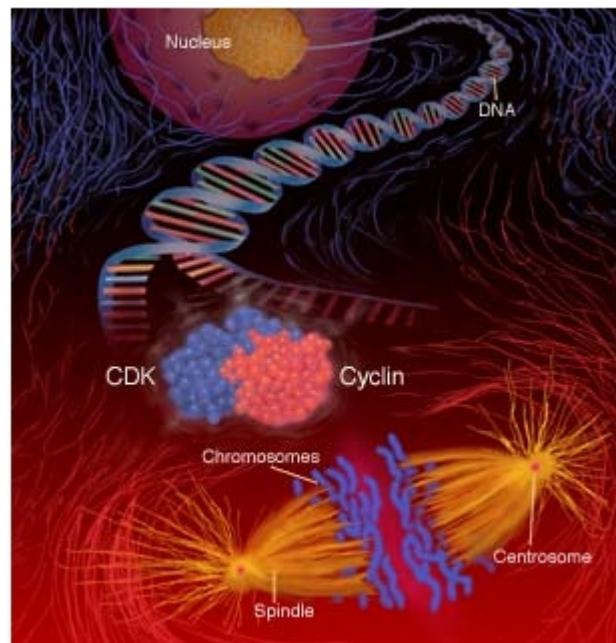
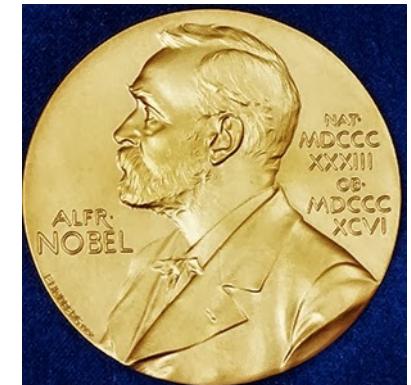
S - DNA synthesis

G2 - Growth and preparation for mitosis

M - Mitosis (cell division)

INTRODUCTION: NOBEL PRIZE IN MEDICINE 2001

- “Key regulators of the cell cycle”
- Using genetic and biochemical methods CDK and cyclin were identified

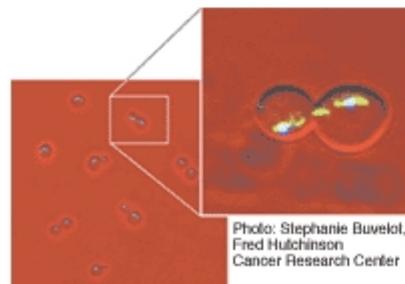


INTRODUCTION: NOBEL PRIZE IN MEDICINE 2001

- 1970-1971: CDC (cell division cycle genes) in yeast
- CDC28: control of the cell cycle start
- “Checkpoints” for the cell cycle control
- Correct order in the cell cycle phases



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

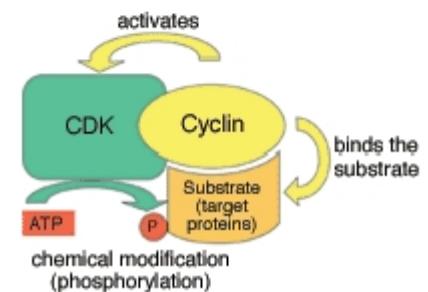


INTRODUCTION: NOBEL PRIZE IN MEDICINE 2001

- 1976-1980: cdc2 – regulator of the cell cycle in yeast
- CDK1 (cyclin dependent kinases)



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

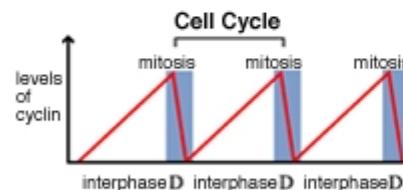


INTRODUCTION: NOBEL PRIZE IN MEDICINE 2001

- 1982: cyclins discovery in sea urchin
- Their degradation during mitosis



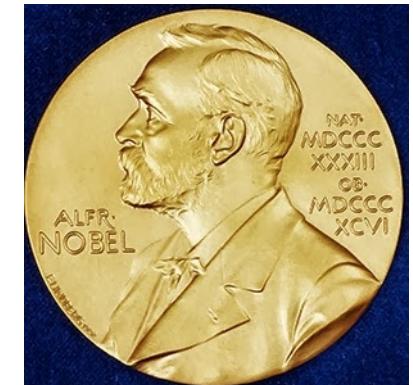
Tim Hunt,
born 1943,
Imperial Cancer
Research Fund,
Clare Hall
Laboratories,
South Mimms,
UK.



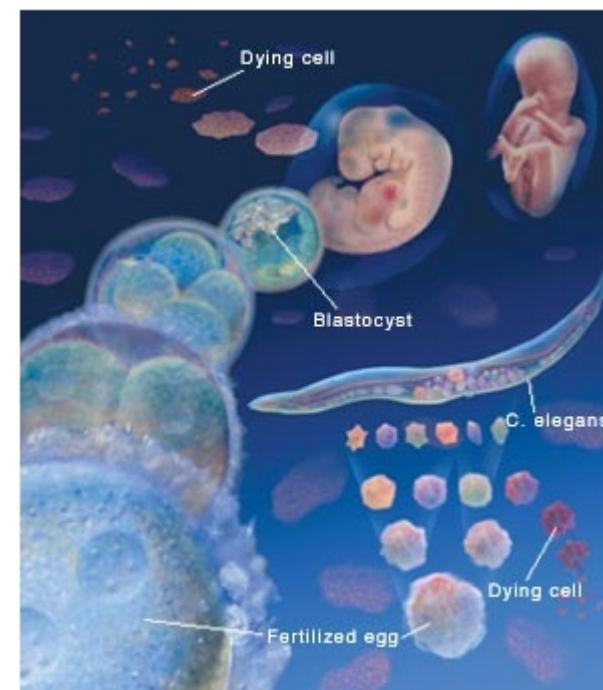
Photos: Eva Löfman,
Carl Löfman

INTRODUCTION: NOBEL PRIZE IN MEDICINE 2002

- “Genetic regulation of organ development and programmed cell death”
- Key genes were identified in *C. elegans*



C. elegans

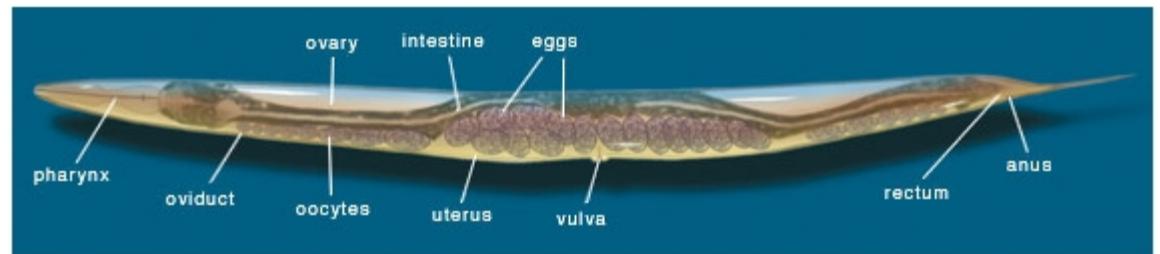


INTRODUCTION: NOBEL PRIZE IN MEDICINE 2002

- Early 1960s: established *C. elegans* as a model organism for cell differentiation and organ development studies



**Sydney
Brenner**, born
1927,
La Jolla, CA,
USA.

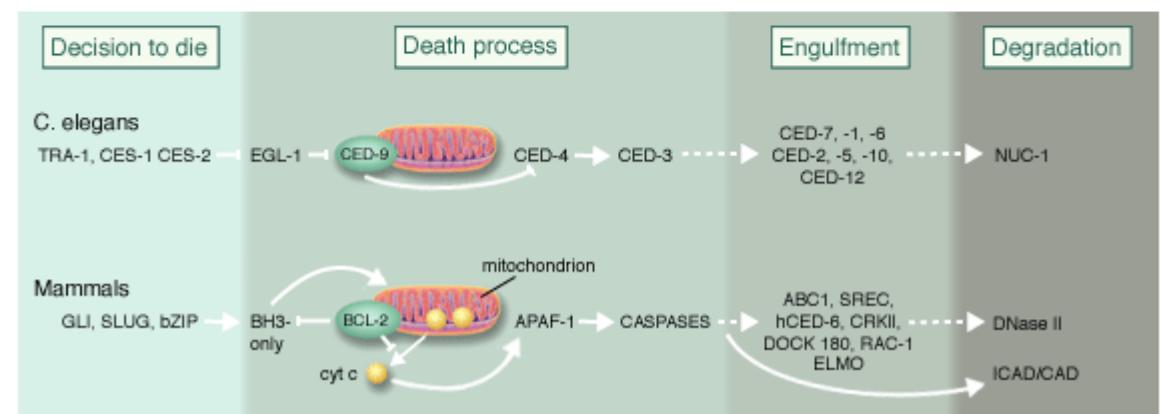


INTRODUCTION: NOBEL PRIZE IN MEDICINE 2002

- 1986: identification of first two “death genes: *ced3*, *ced4*
- Identification of *ced-9*: protection against the cell death



Robert Horvitz,
born 1947,
Cambridge, MA,
USA.

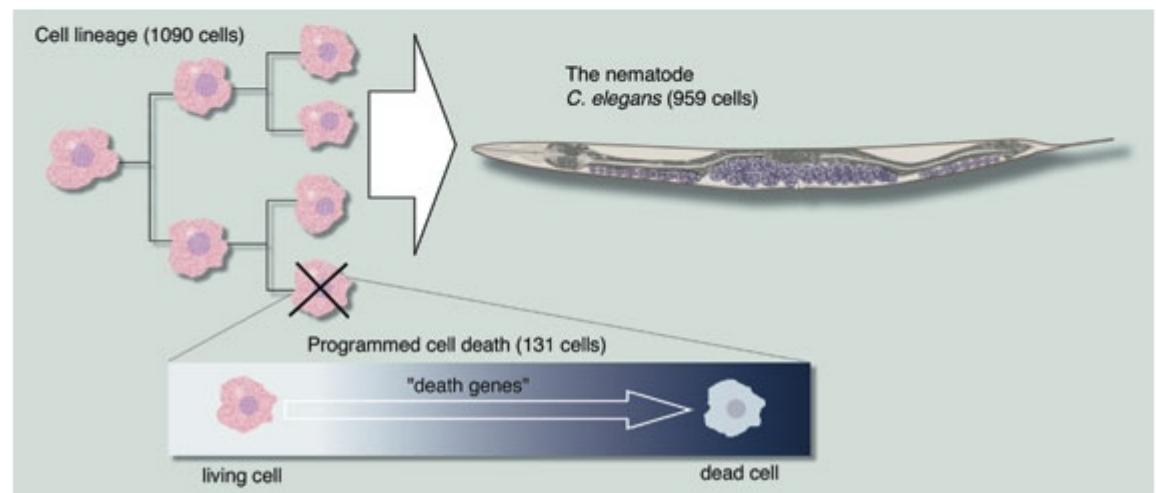


INTRODUCTION: NOBEL PRIZE IN MEDICINE 2002

- 1976: all nematode cells undergo the same path of cell division and differentiation
- Specific cells always die by programmed cell death
- Nuc-1 gene discovery

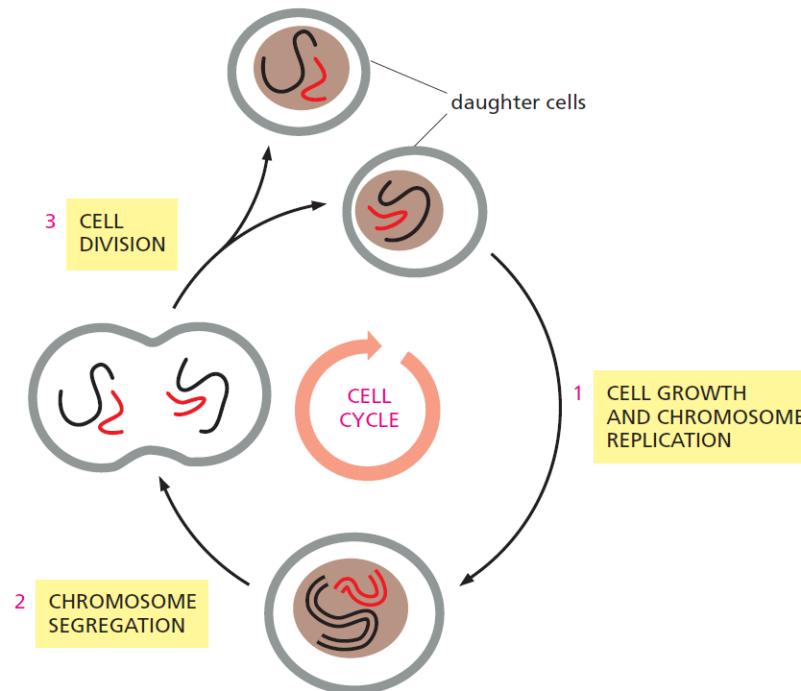


John Sulston,
born 1942,
Cambridge,
England.



THE CELL DOCTRINE

“Where a cell arises, there must be a previous cell, just as animals can only arise from animals and plants from plants”, Rudolph Virchow (1858).



- How do cells duplicate their contents?
- How do they partition the duplicated contents and split in two?
- How do they coordinate all the machinery that is required for these two processes?

TIME SCALES OF CELL CYCLE

➤ Cell cycle length are highly specific:

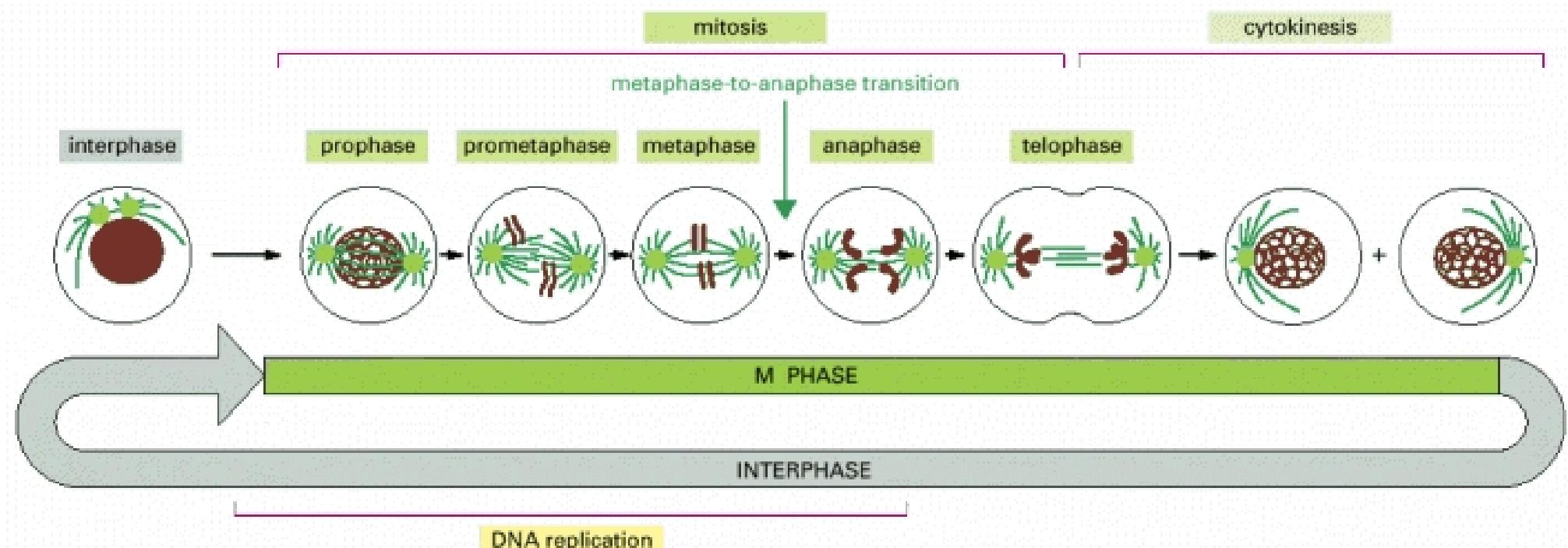
- organism

- cell type

CELL TYPE	CELL-CYCLE TIMES
Early frog embryo cells	30 minutes
Yeast cells	1.5–3 hours
Mammalian intestinal epithelial cells	~12 hours
Mammalian fibroblasts in culture	~20 hours
Human liver cells	~1 year

BASIC FUNCTION OF THE CELL CYCLE

- Accurate duplication of DNA in chromosomes
- Segregation of the copies precisely into daughter cells
- High conservation



Processes observed in the microscope

PHASES OF THE CELL CYCLE

➤ M-phase (seen in microscope)

- mitosis (division of nucleus)

- cytokinesis (splitting into two cells)

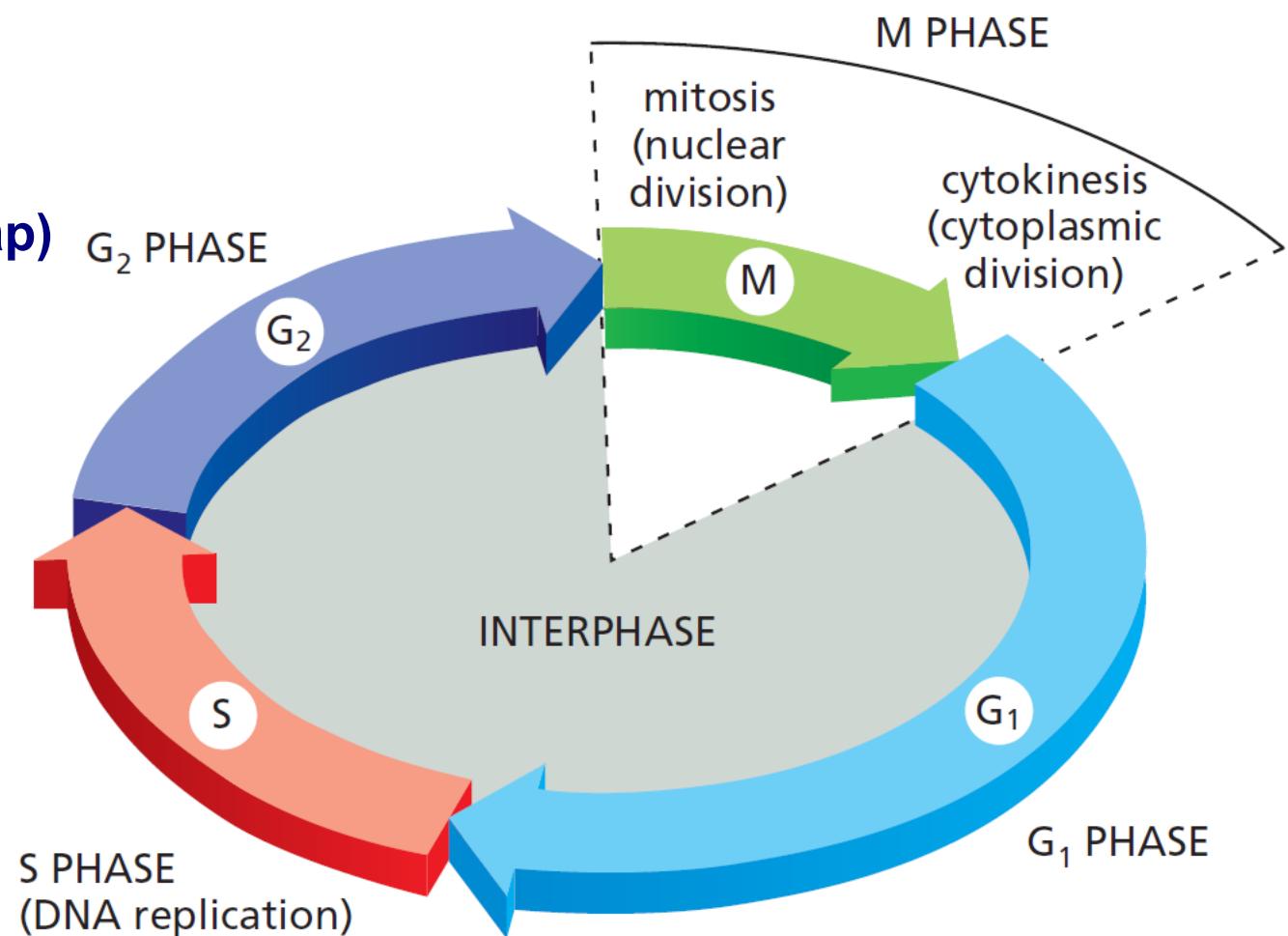
➤ Interphase:

- S-phase (S-synthesis)

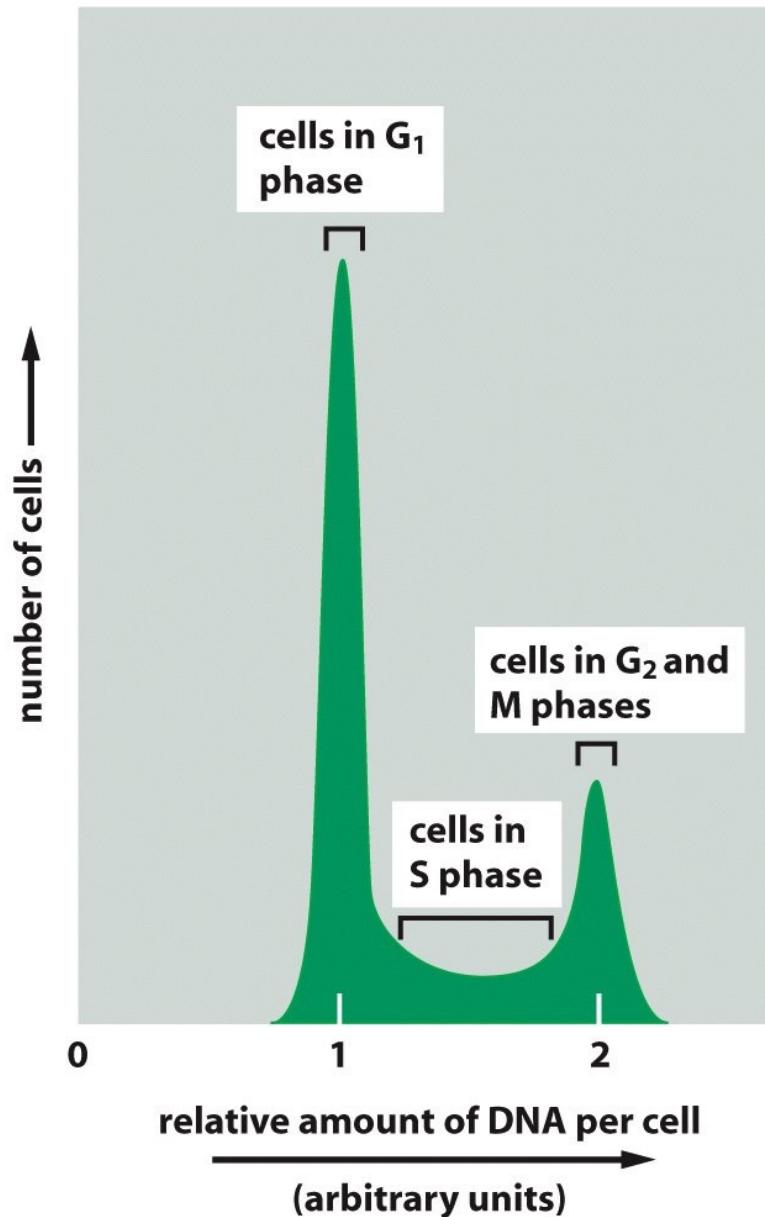
- G₁-phase

- G₂-phase

(G-gap)

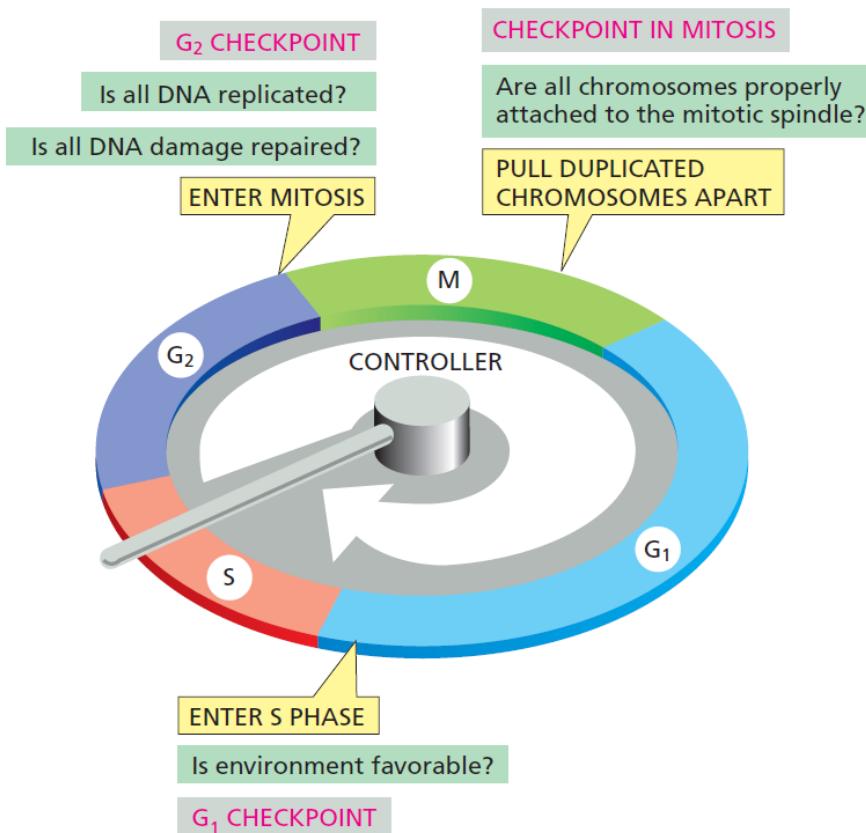


AMOUNT OF DNA PER CELL: FLOW CYTOMETRY



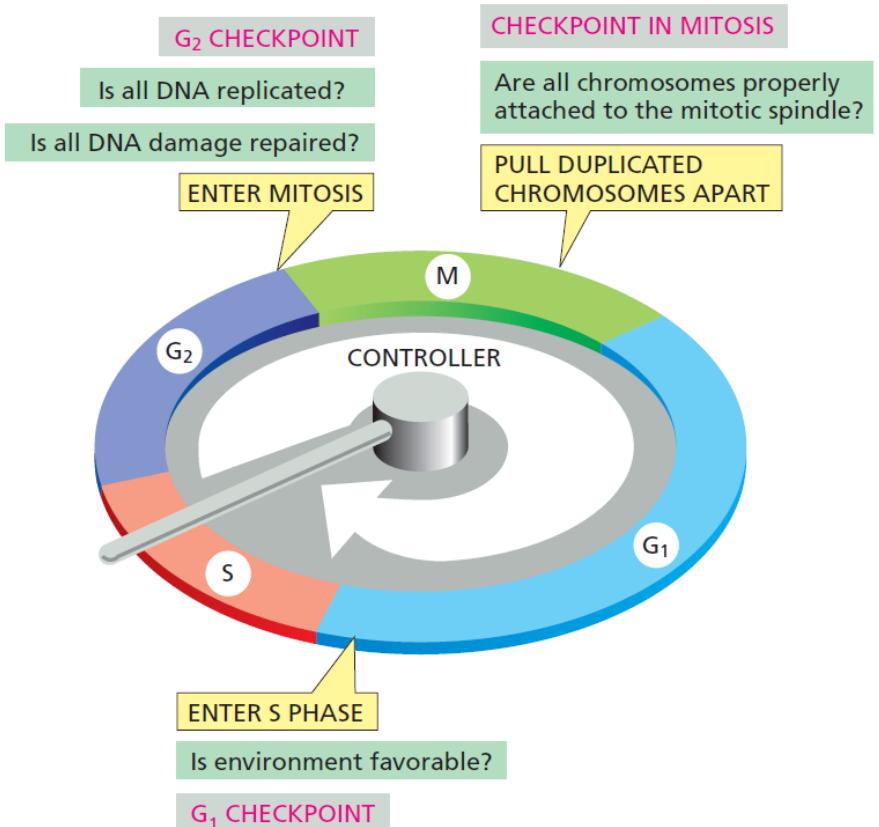
CELL CYCLE CONTROL SYSTEM

- Function: control of proper sequence of events
- Critical checkpoints where the cell cycle can stop:
 - G₁ (option for G₀)
 - G₂
 - M



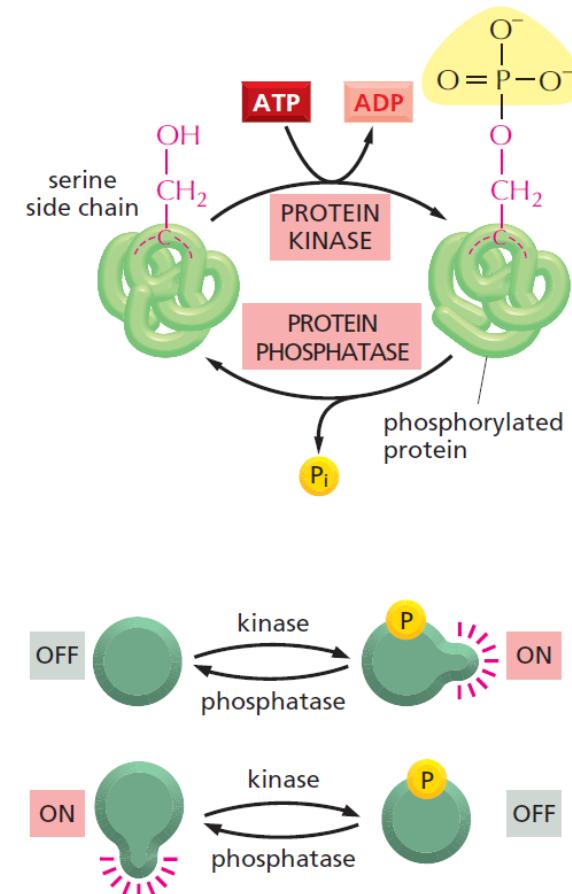
CELL CYCLE CONTROL SYSTEM

- Each event starts at the specific time
- Each event has a correct order
- Each event occurs only once per cycle
- Binary regulation: complete/irreversible order
- Compensatory mechanisms
- Adaptability
- Feedback principle
 - f.i.: DNA is synthesized slowly =>
=> mitosis is delayed)

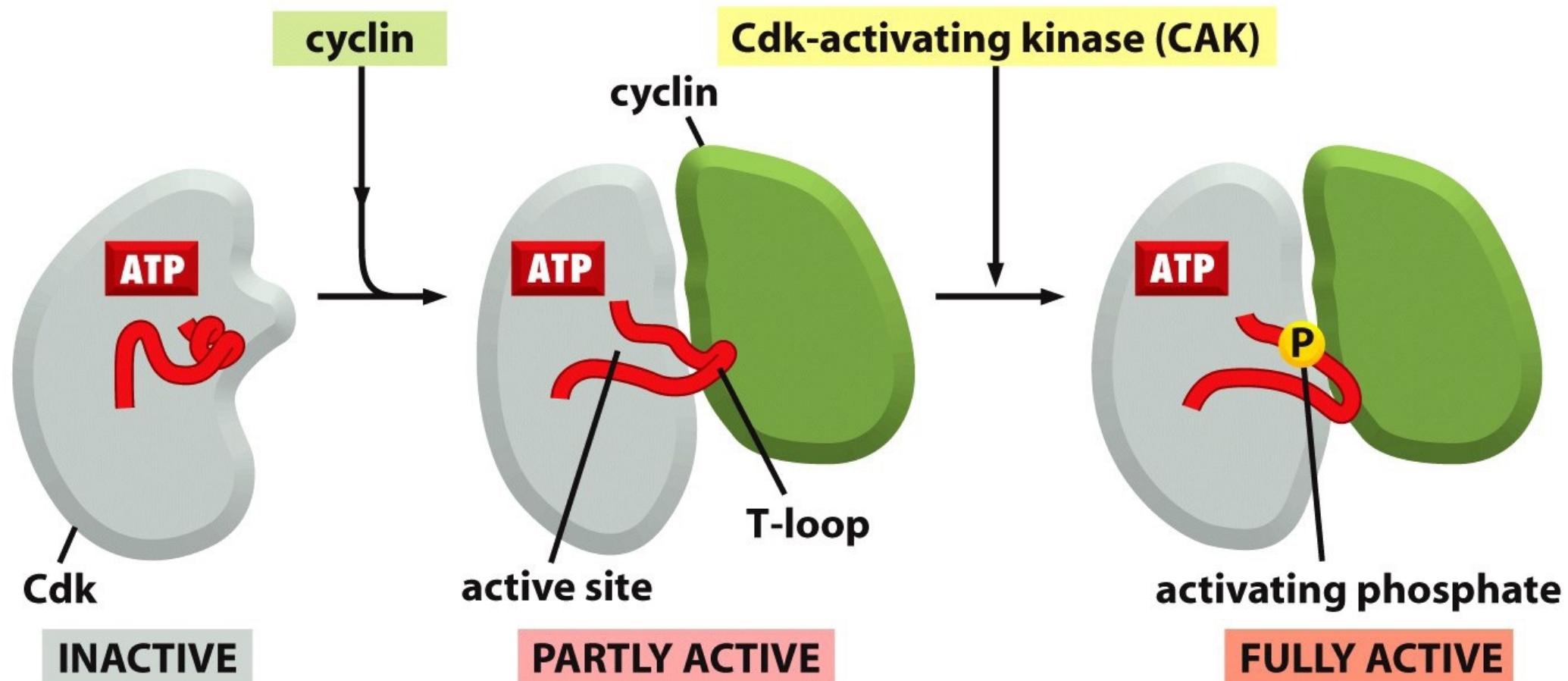


CELL CYCLE CONTROL SYSTEM

- Machinery of two types is switched on and off:
 - production of new components
 - proper placement of these components in the division process
- Cdks (cyclin dependent protein kinases)/cyclin interactions:
 - phase specific
 - cyclic
 - key role of phosphorylation

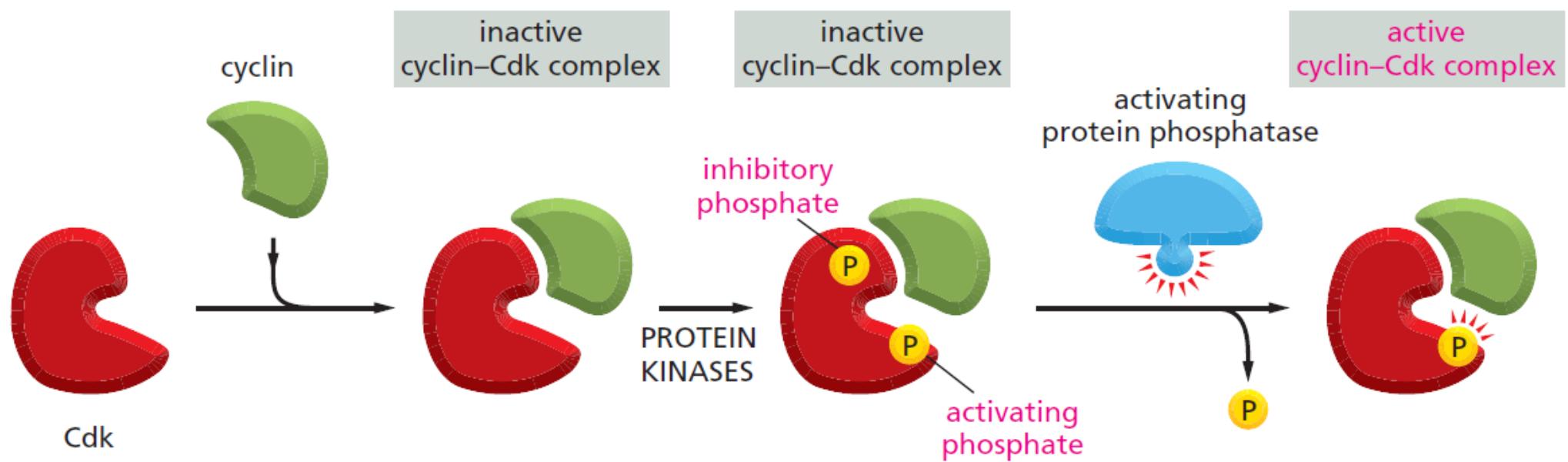


CELL CYCLE CONTROL SYSTEM: CDK/CYCLIN ACTIVATION

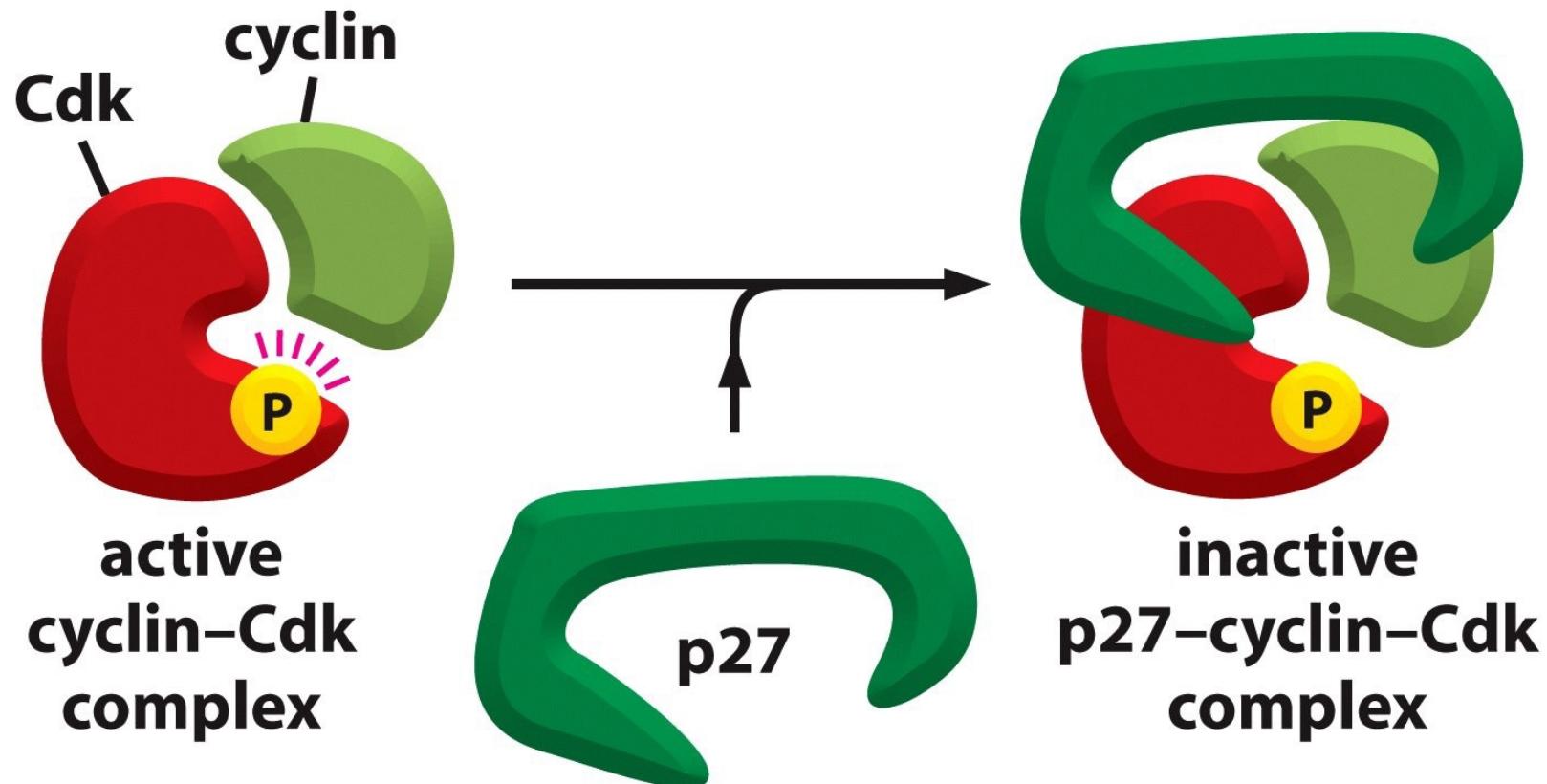


CELL CYCLE CONTROL SYSTEM: CDK/CYCLIN ACTIVATION

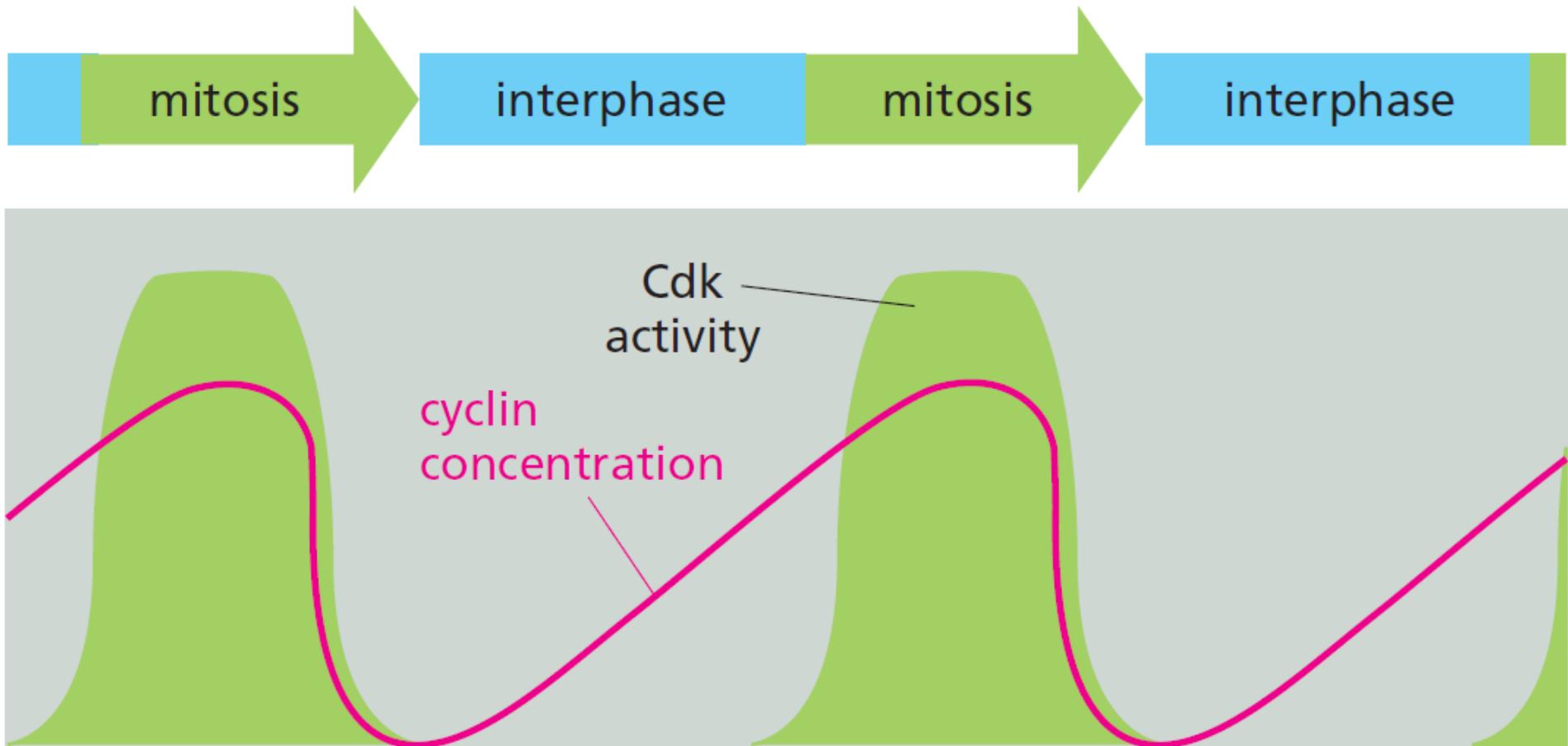
- Two sites of phosphorylation (Wee1 kinase) => inhibition
- One site of phosphorylation (Cdc25 phosphatase)=> activation



CELL CYCLE CONTROL SYSTEM: CDK INHIBITORY KINASES



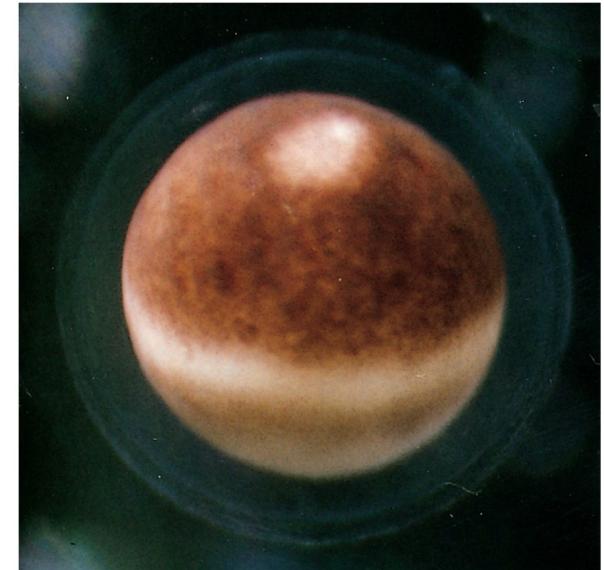
CELL CYCLE CONTROL SYSTEM: CYCLIN CONCENTRATION



DISCOVERY OF CYCLINS AND CDKS

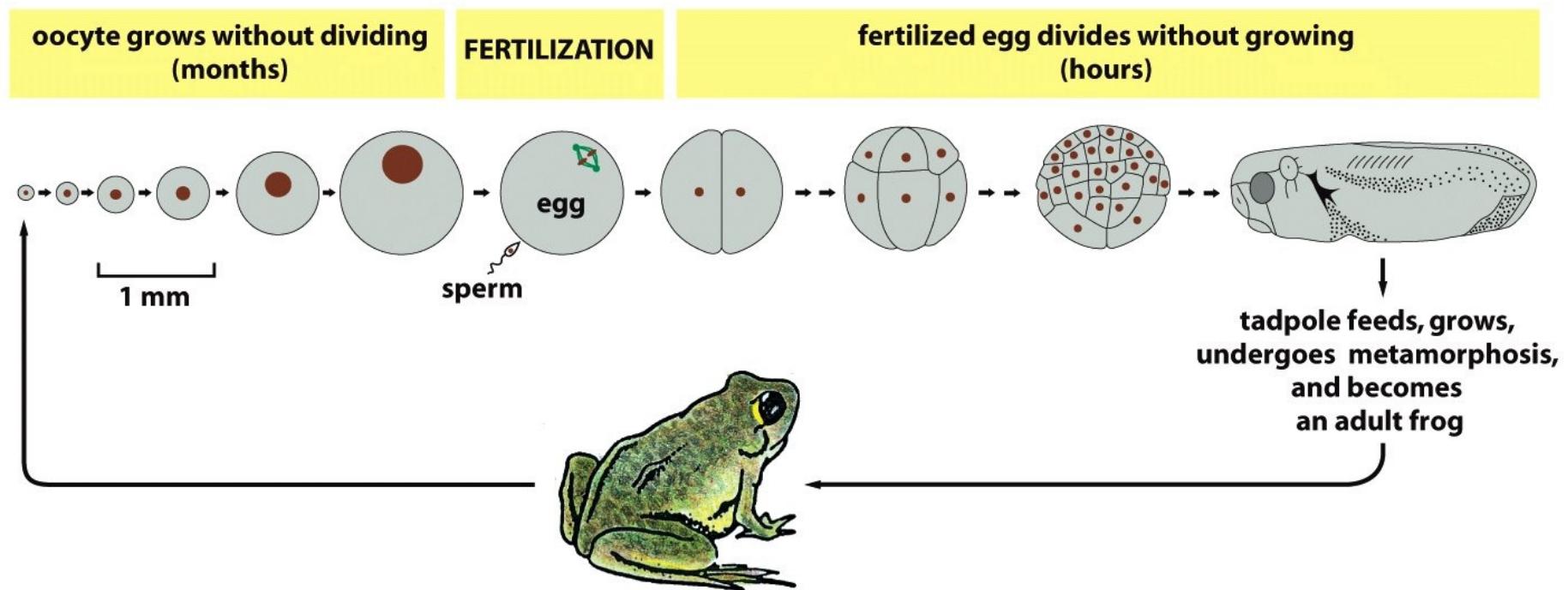
➤ *Xenopus* egg:

- large
- many divisions (S and M)
- almost no G phases (no mRNA transcription)
- extraction => oocyte



0.5 mm

Frog egg

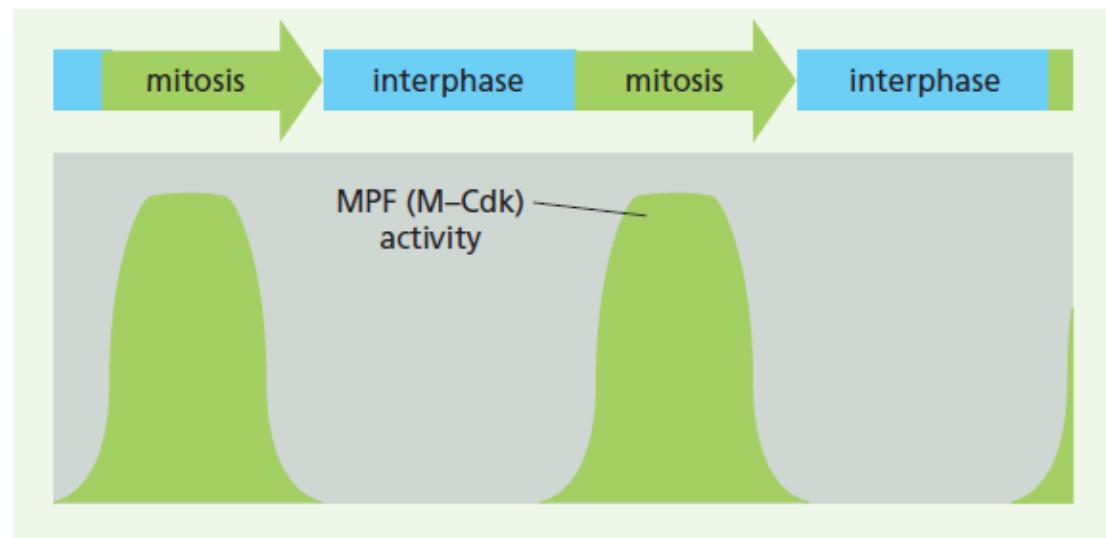
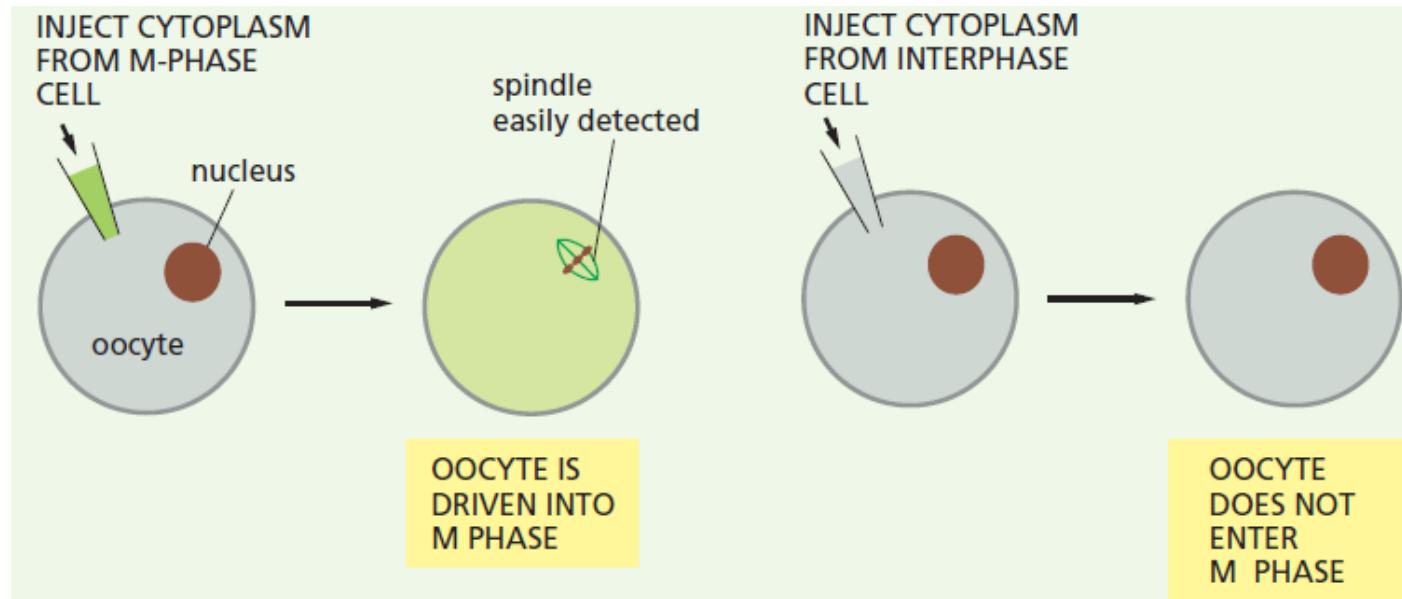


DISCOVERY OF CYCLINS AND CDKS

➤ MPF (maturation promoting factor):

- protein kinase

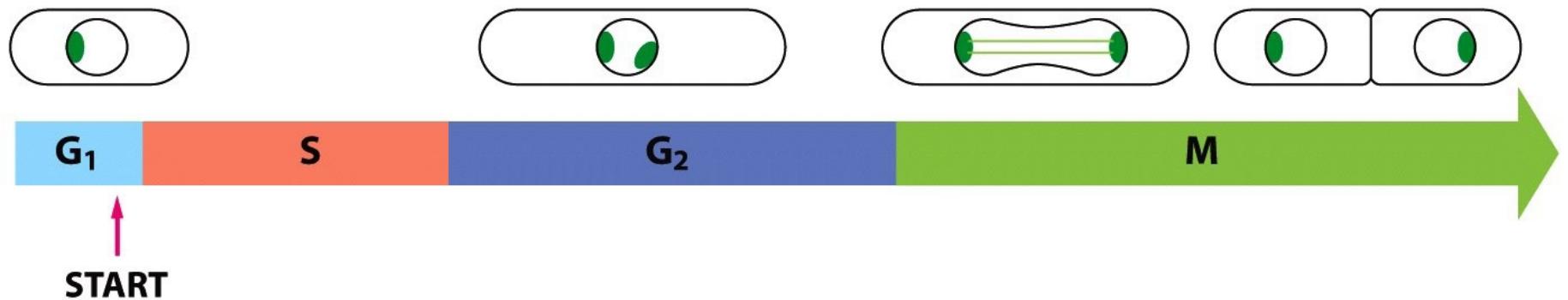
- M-cyclin



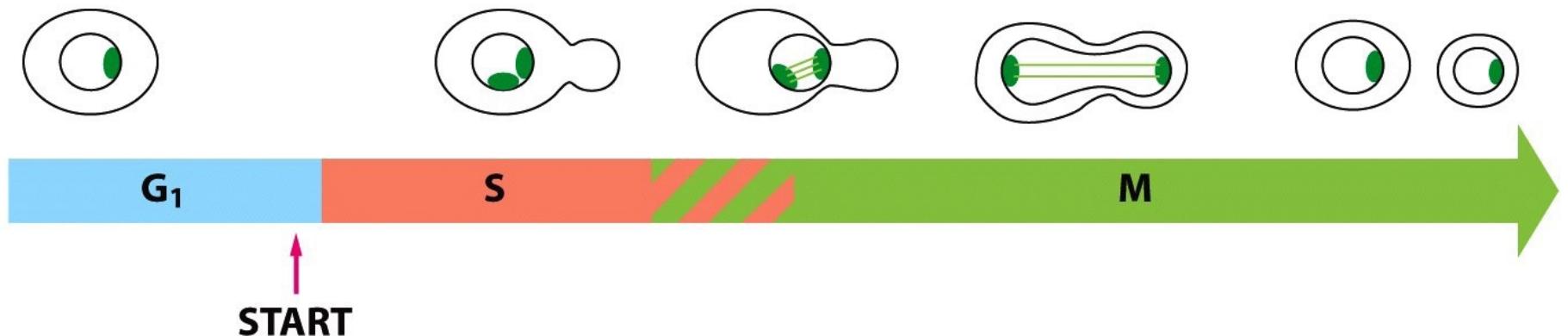
CELL CYCLE STUDIES IN YEAST

- Very fast
- Small genome (~ 1% of human)
- Nuclear envelope does not break in M phase

FISSION YEAST (*Schizosaccharomyces pombe*)



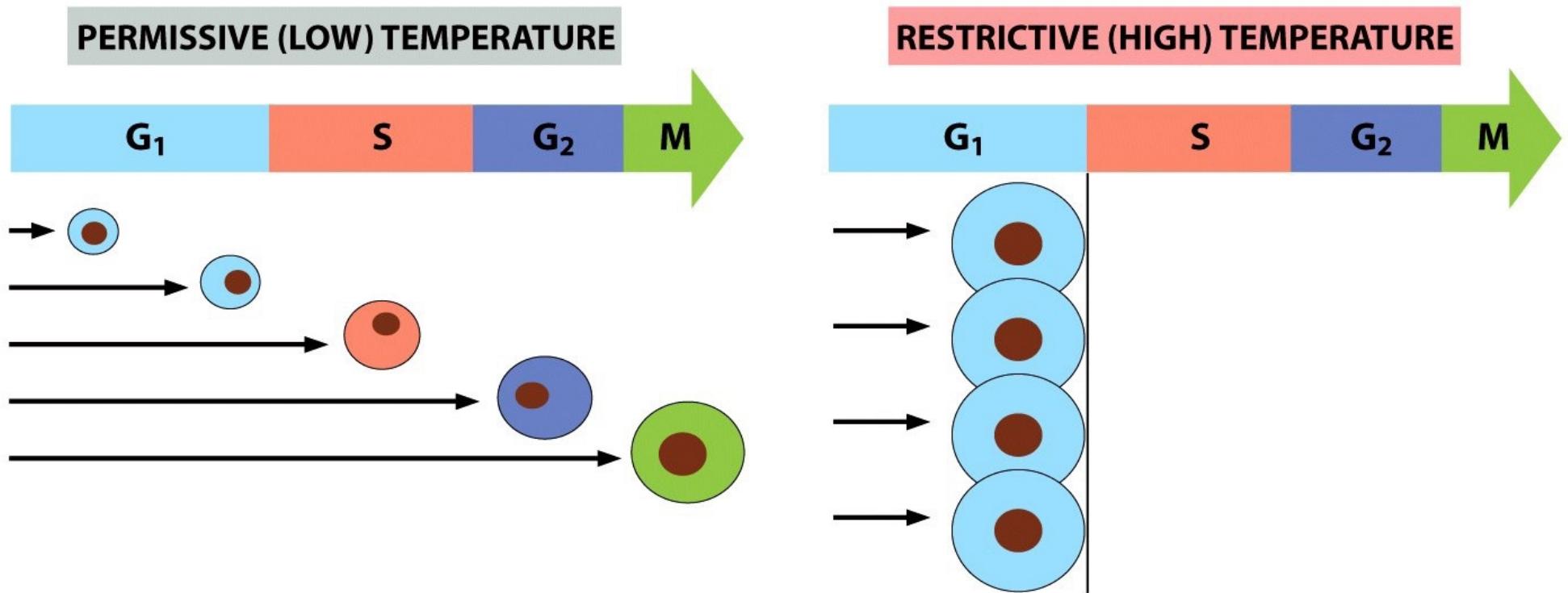
BUDDING YEAST (*Saccharomyces cerevisiae*)



CELL CYCLE STUDIES IN YEAST

➤ Cdc-mutants:

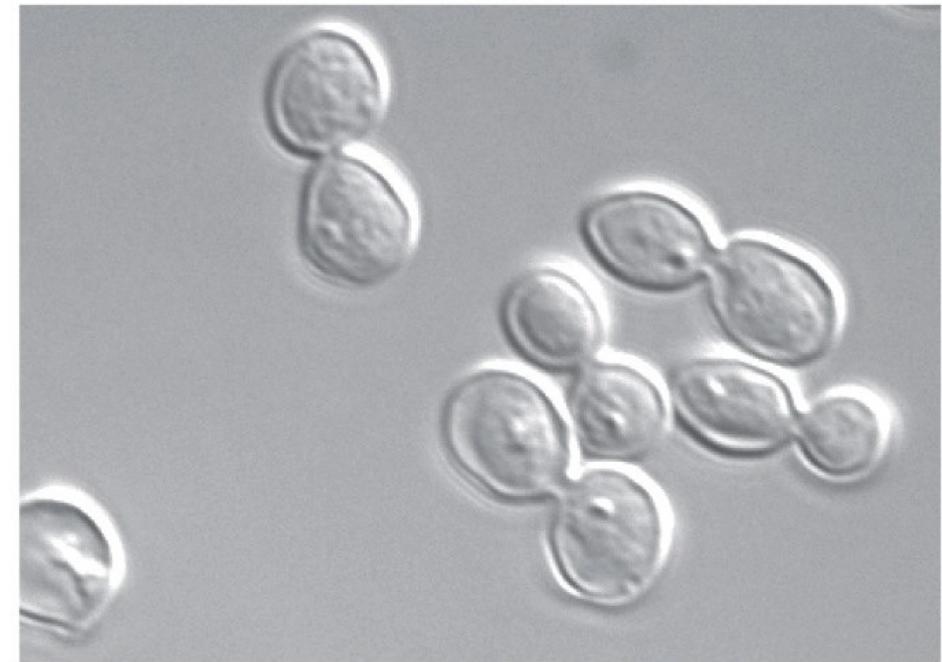
- conditional
- temperature-sensitive (permissive and restrictive conditions)
- arrest in the same point



CELL CYCLE STUDIES IN YEAST



WT

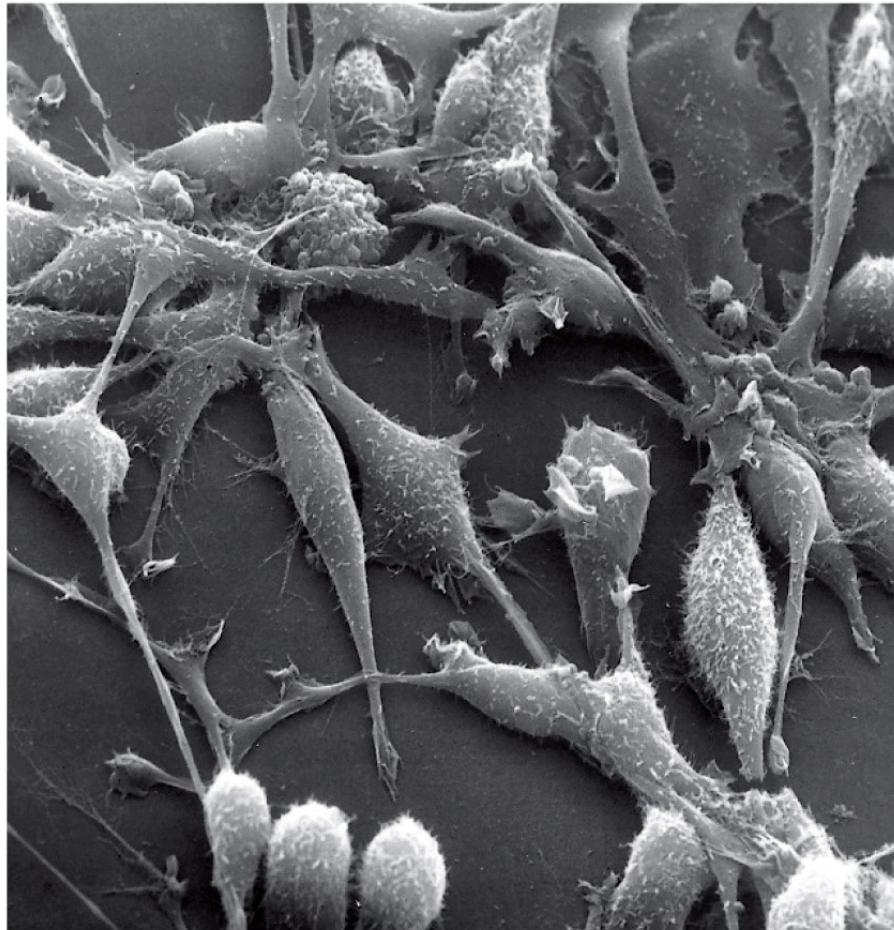


cdc15 mutant arrest

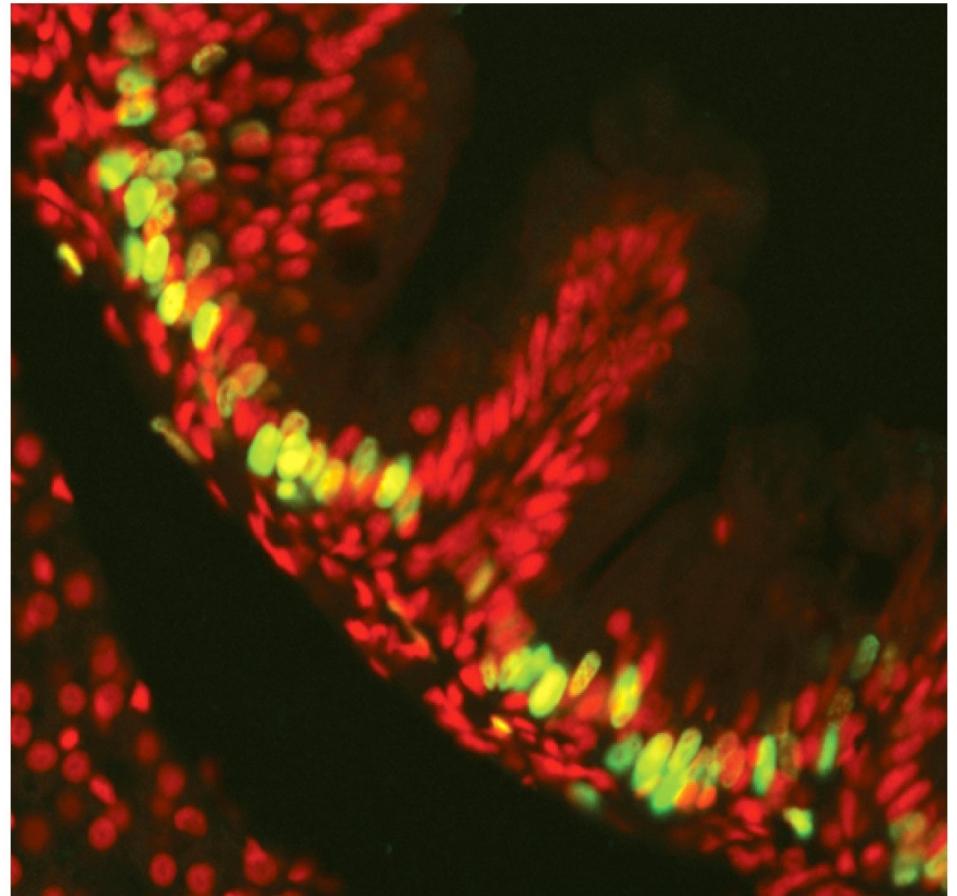
20 μ m

CELL CYCLE STUDIES IN MAMMALIAN CELLS

- Isolated cells from tissues and tumors
- Essential nutrients and other factors
- “Immortalized” cell lines

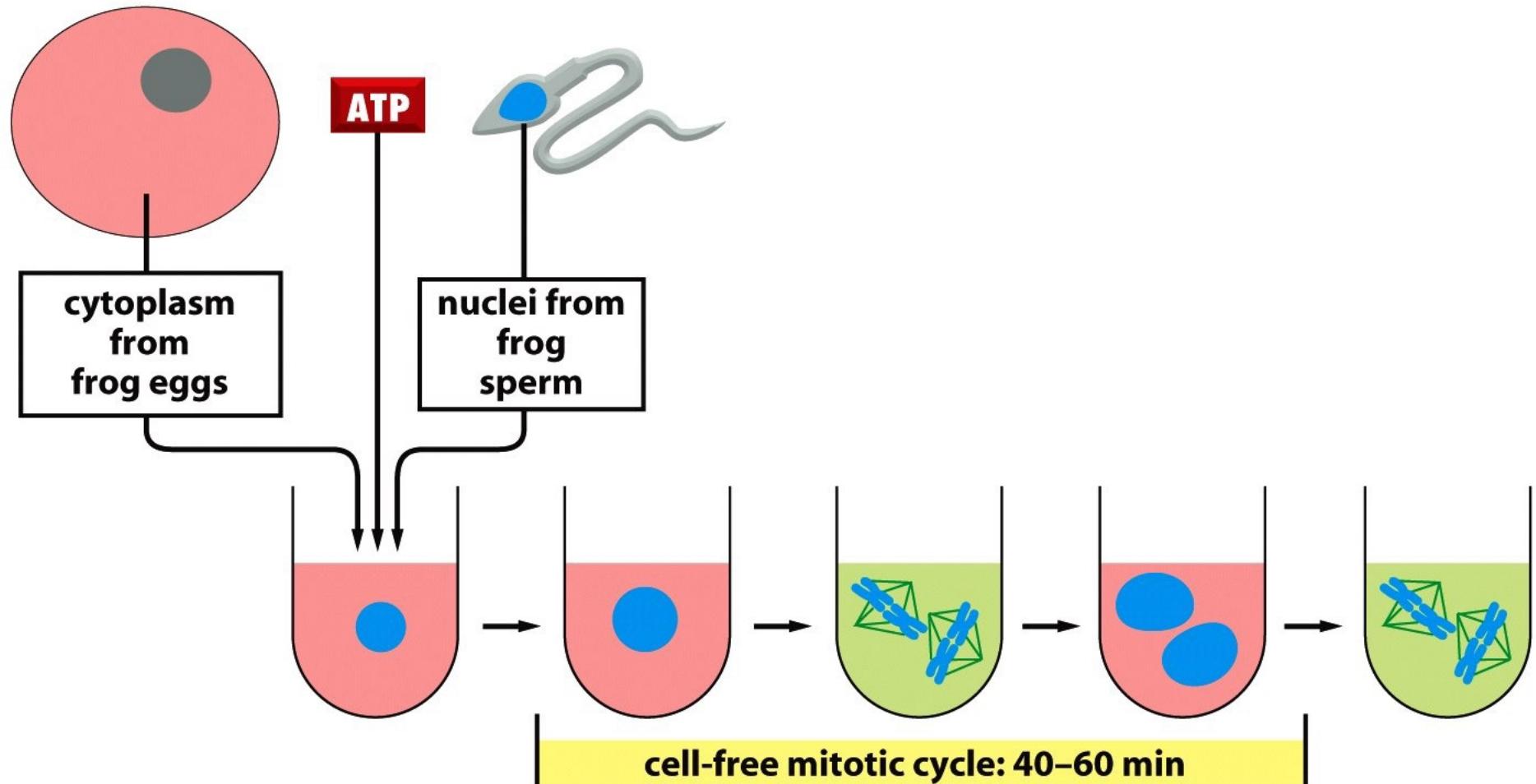


10 μm

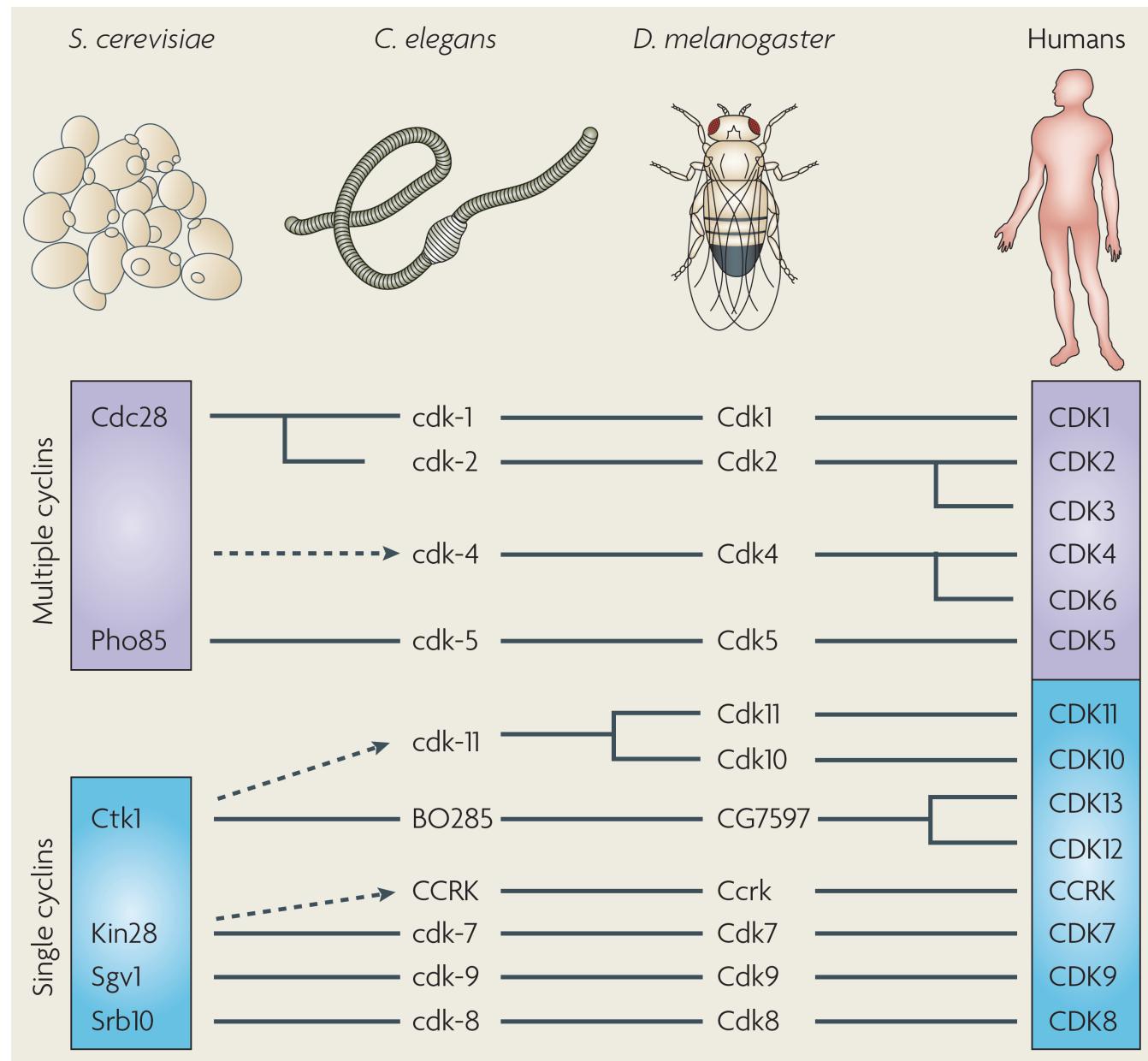


S-phase labelling

CELL CYCLE STUDIES IN CELL-FREE SYSTEM



EVOLUTION OF CELL CYCLE PROTEINS

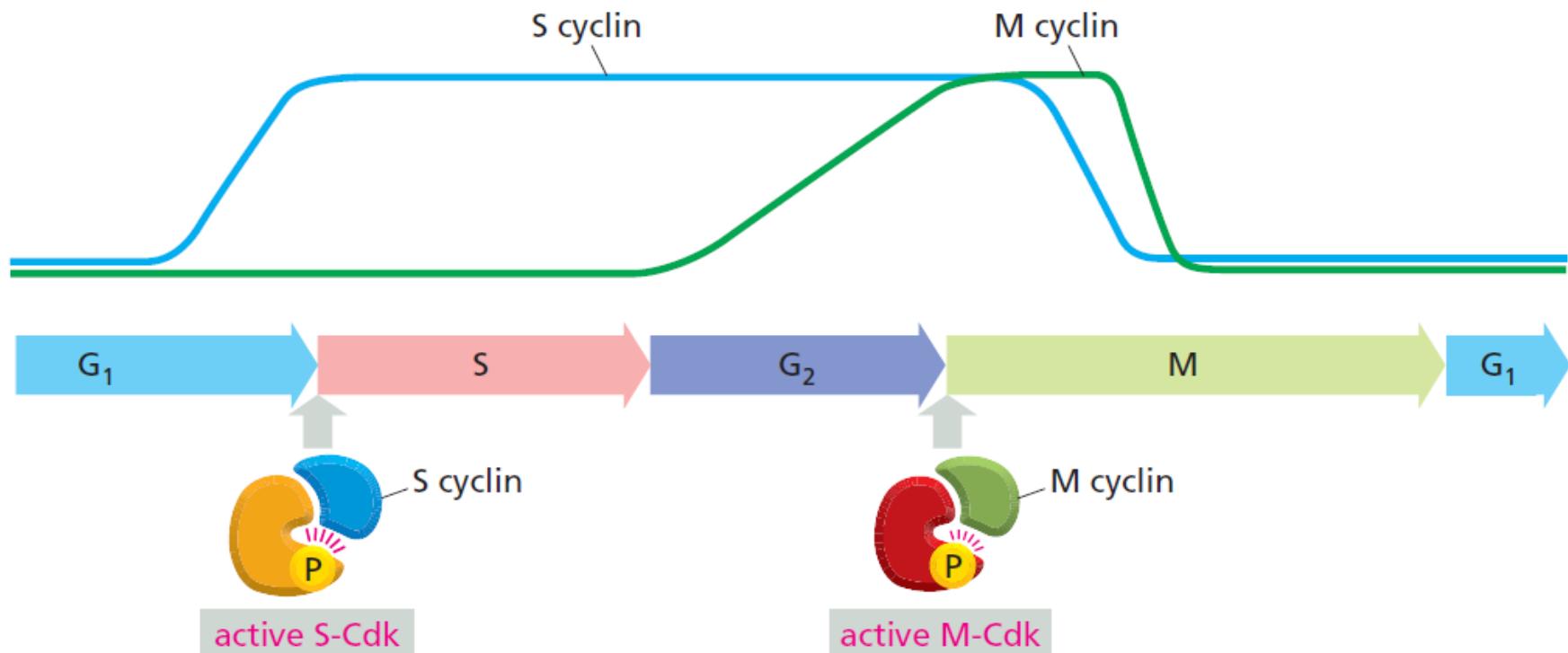


CYCLINS SPECIFICITY

CYCLIN-CDK COMPLEX	CYCLIN	CDK PARTNER
G ₁ -Cdk	cyclin D*	Cdk4, Cdk6
G ₁ /S-Cdk	cyclin E	Cdk2
S-Cdk	cyclin A	Cdk2
M-Cdk	cyclin B	Cdk1**

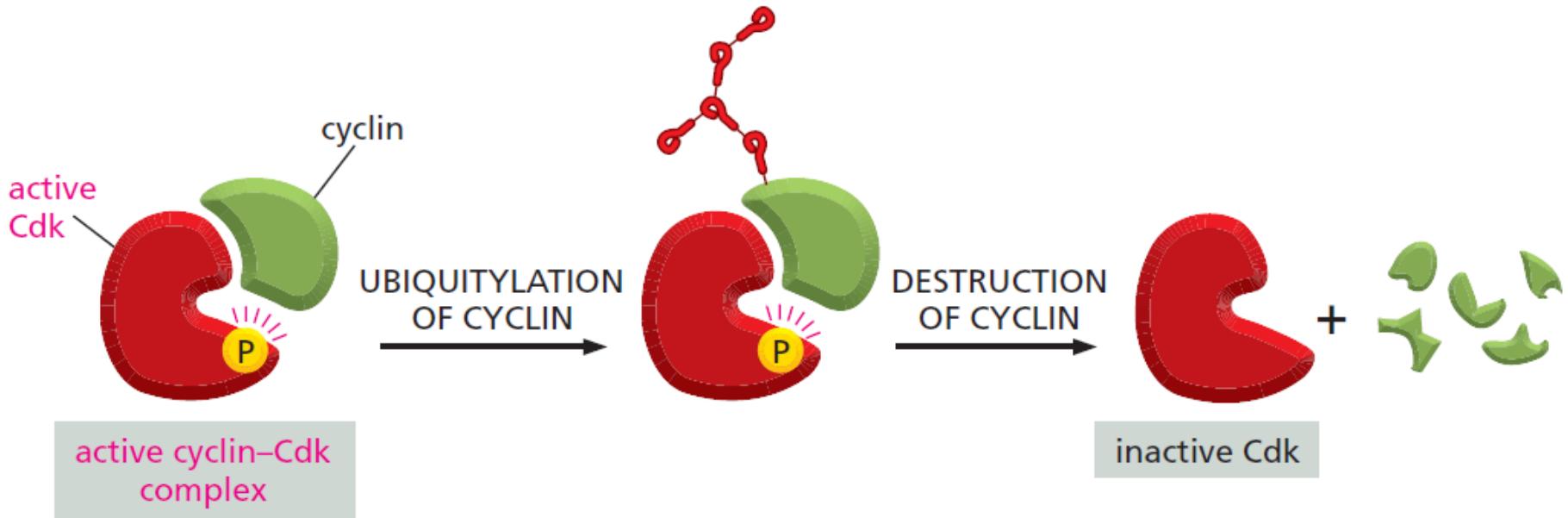
*There are three D cyclins in mammals (cyclins D1, D2, and D3).

**The original name of Cdk1 was Cdc2 in vertebrates.



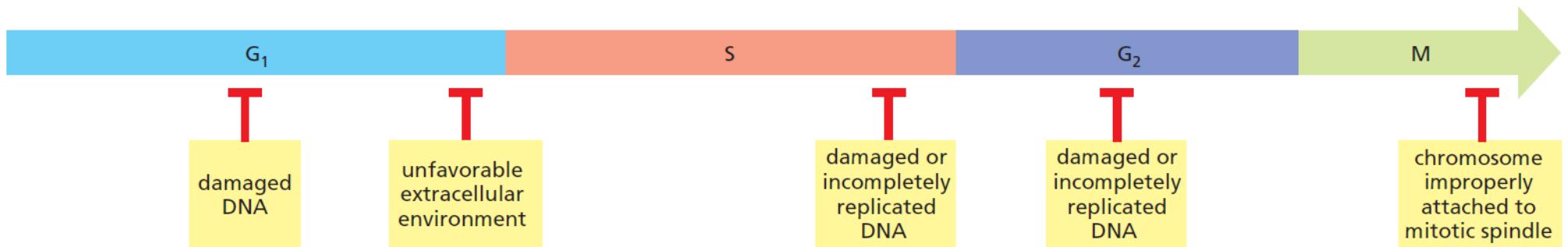
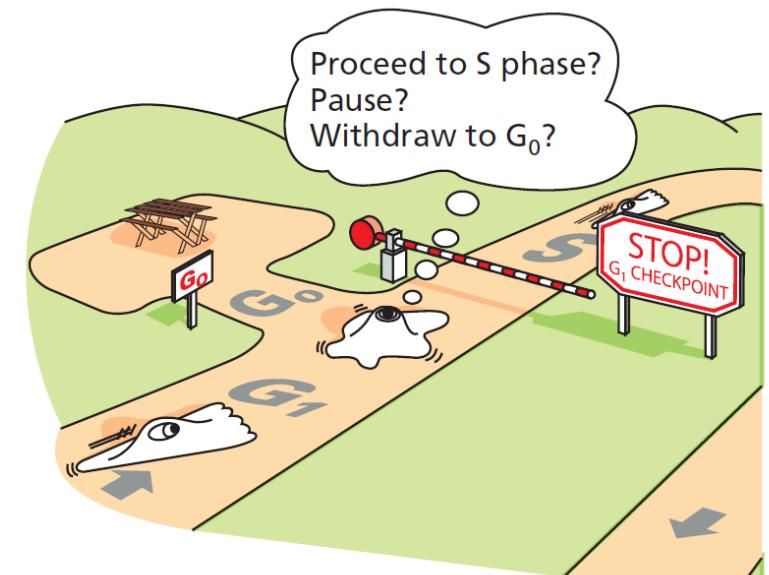
CELL CYCLE CONTROL SYSTEM: CYCLICAL PROTEOLYSIS

- Ubiquitin-mediated proteolysis (L23-24) => lysosomes
- Control of specific cyclins concentration



CELL CYCLE CONTROL SYSTEM: ROLE OF CHECKPOINTS

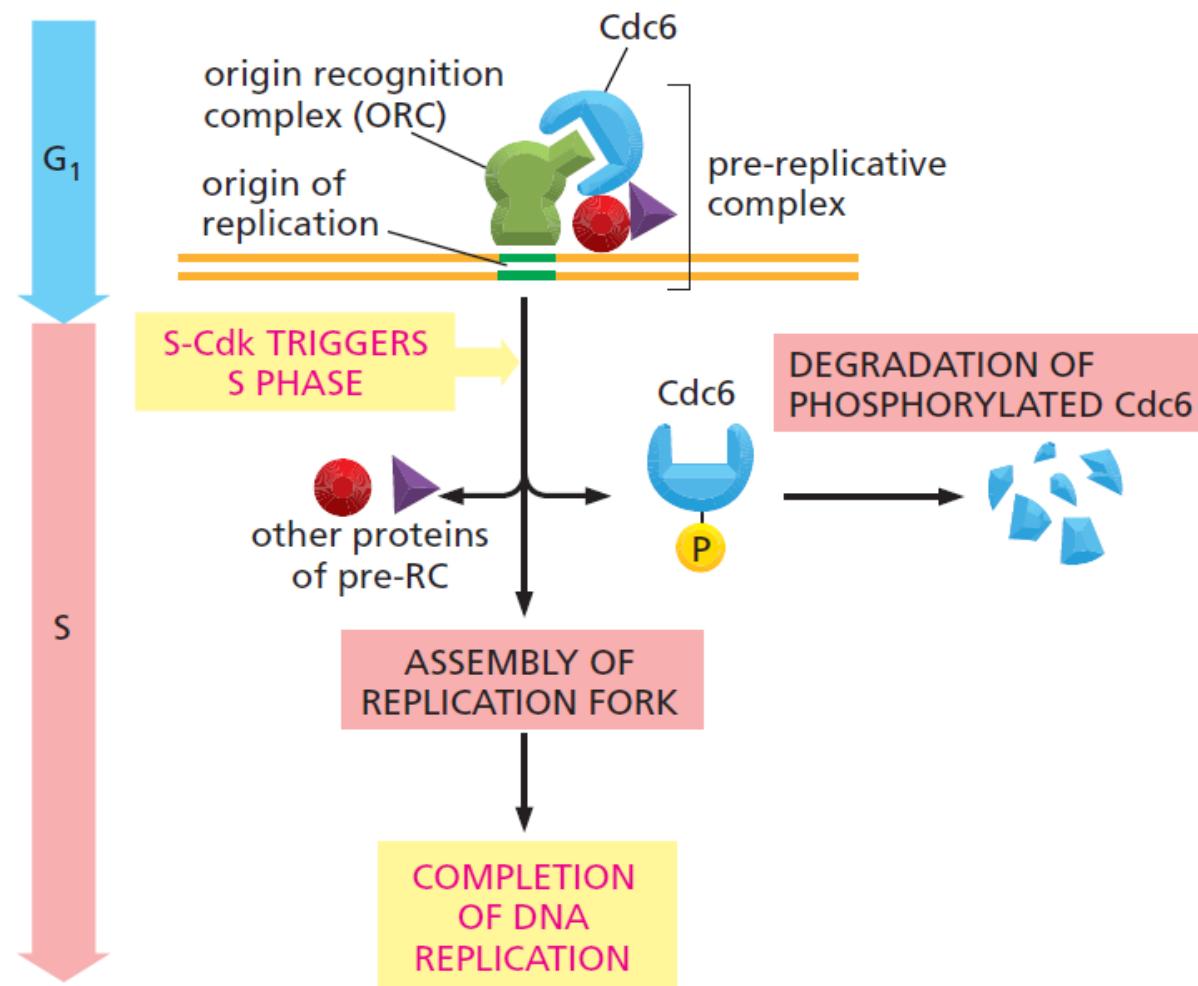
- Molecular brakes: Cdk inhibitor proteins
- Mitogens: extracellular signals (G1 without them)
- “Hungry” cell: G0
- Cell-division diversity: G1 and G0 duration
 - liver cells 1-2 a year
 - epithelia cells once per 12-24 hours
- Irreversible G0 withdrawing
- Transcriptional regulation (oscillation of ~10% of mRNA)



S PHASE

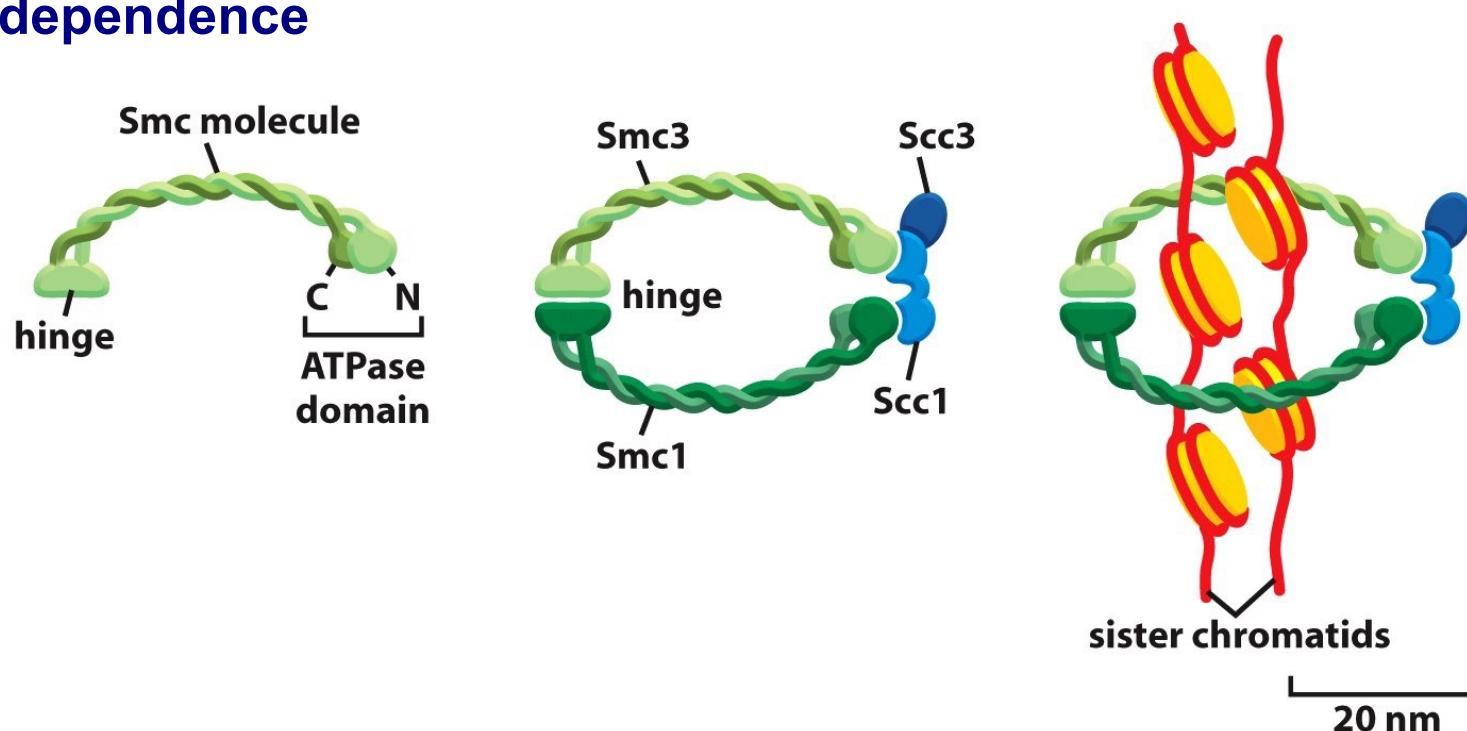
➤ S-Cdk:

- initiation of DNA replication
- blocking re-replication



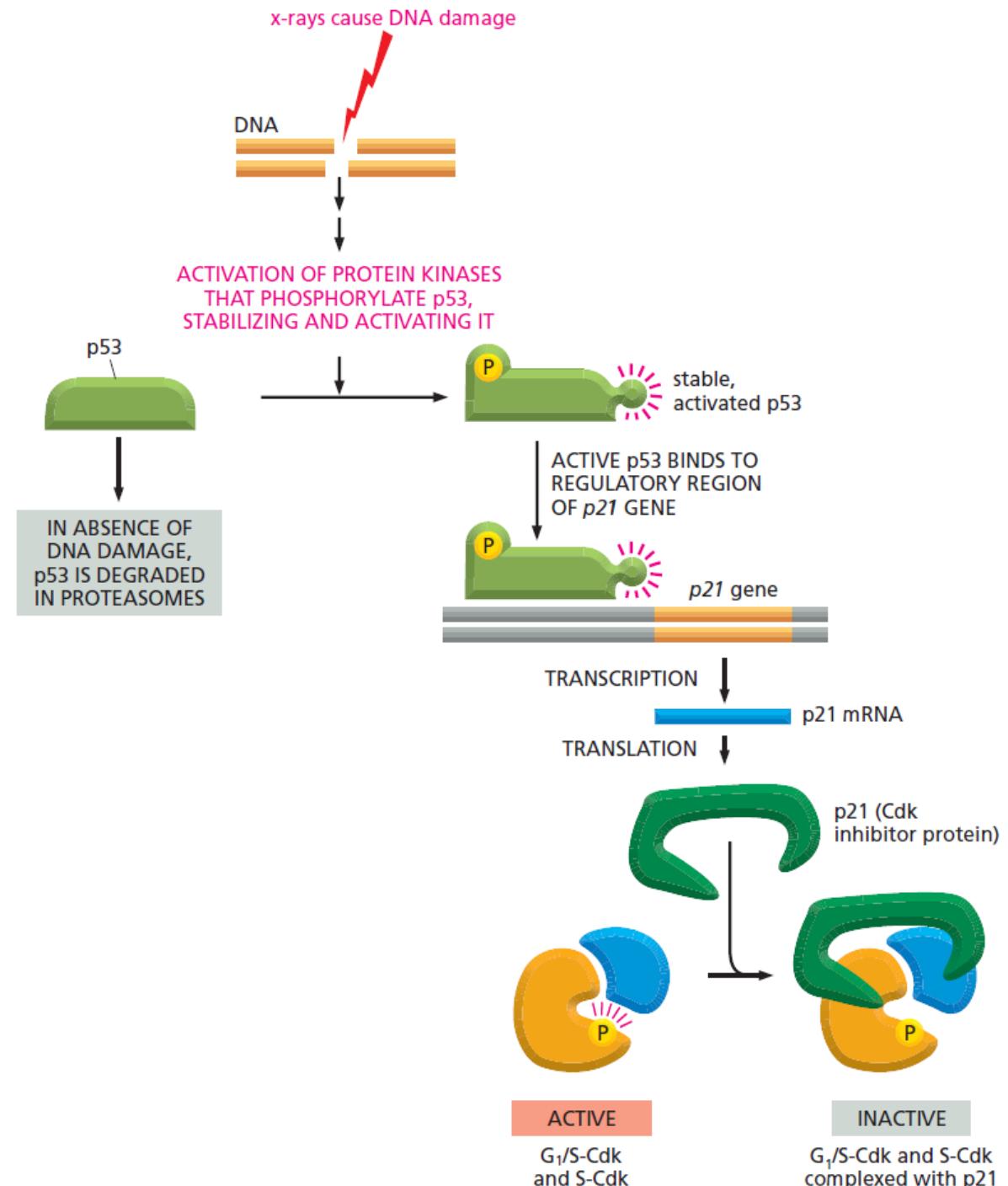
COHESIN: COMPLEX WITH SISTER CHROMATIDS

- After duplications: 2 copies are tightly hold together
- Cohesins: formation of protein rings:
 - crucial for chromosomes segregation
 - broken completely in late mitosis
 - Smc: structural maintaince of chromosomes family
 - ATP dependence



S PHASE: DNA DAMAGE CHECKPOINTS

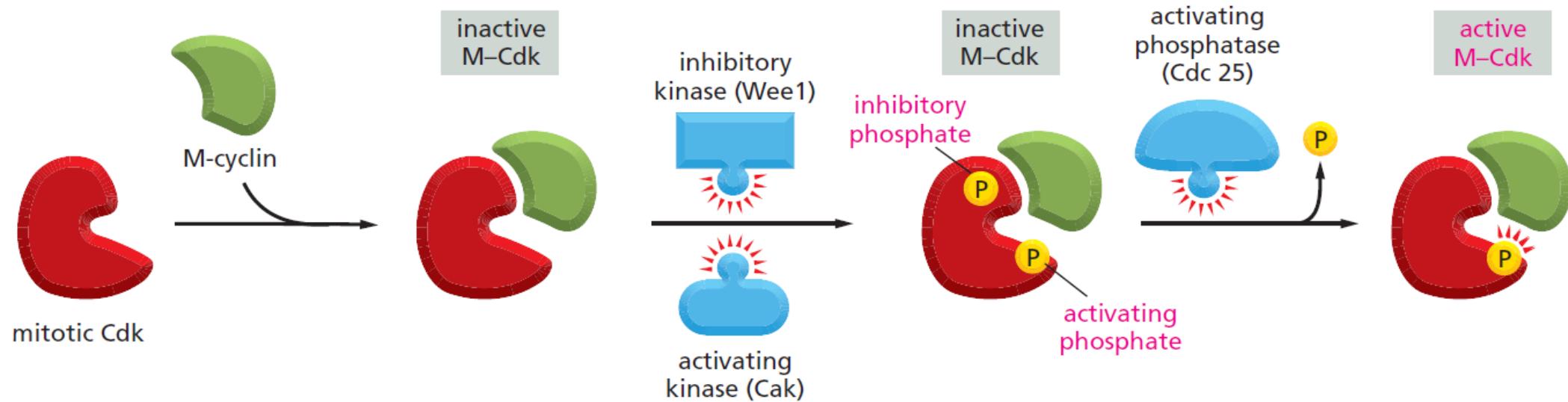
- G1: p53 pathway
- S: through inhibition of activating phosphatases



M PHASE

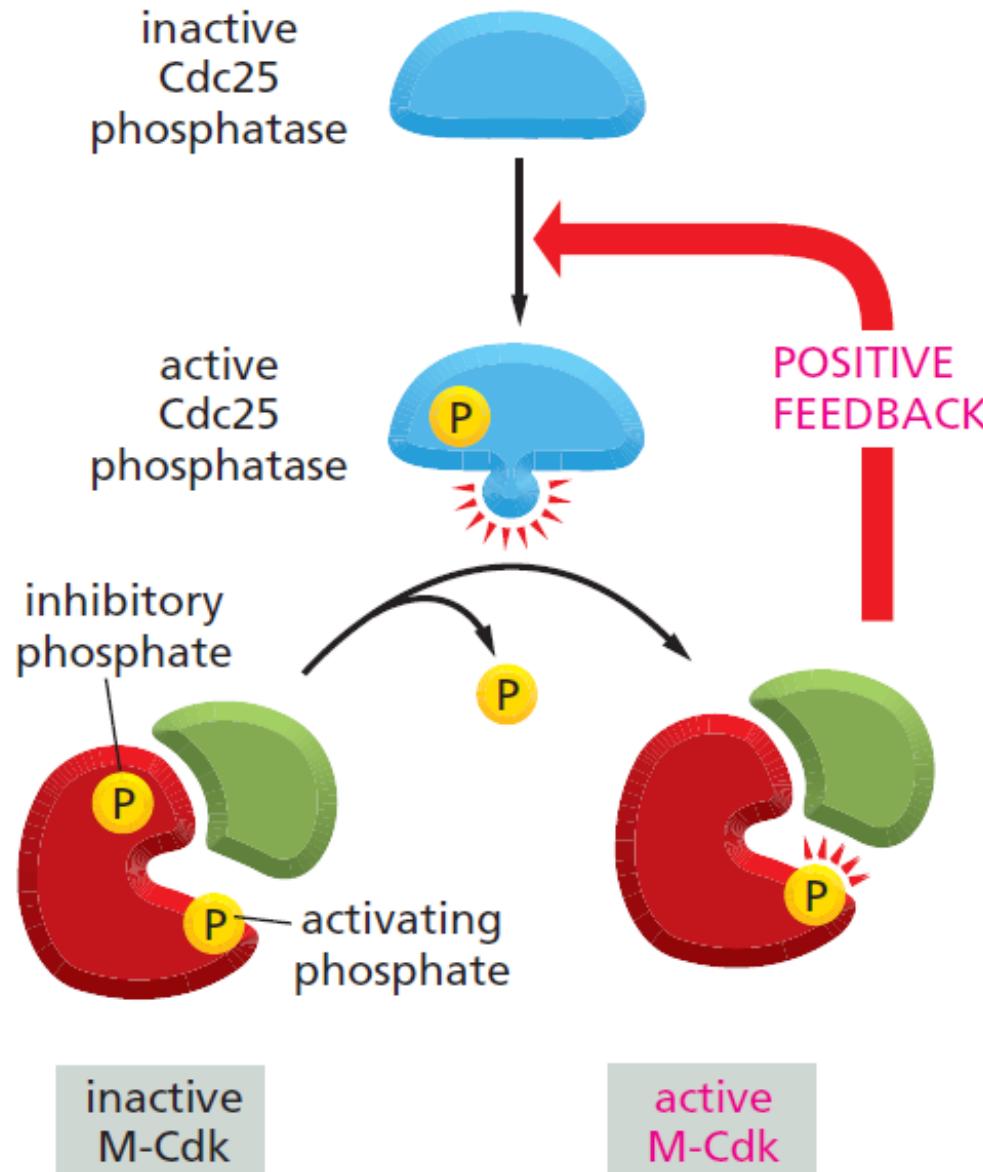
M phase = mitosis + cytokinesis

- Very short part of the cell cycle
- Segregation of chromosomes:
 - assembly of specialized cytoskeletal machine to pull chromosomes apart
 - separation of cytoplasm (cytokinesis)
- M-Cdk:
 - triggers condensation of replicated chromosomes
 - induces assembly of mitotic spindle



M PHASE: M-CDK POSITIVE FEEDBACK LOOP

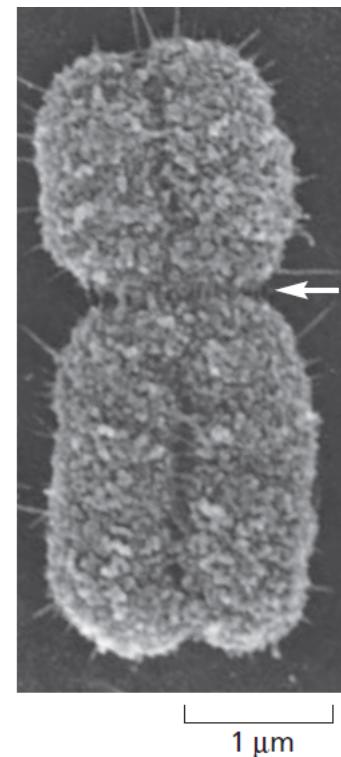
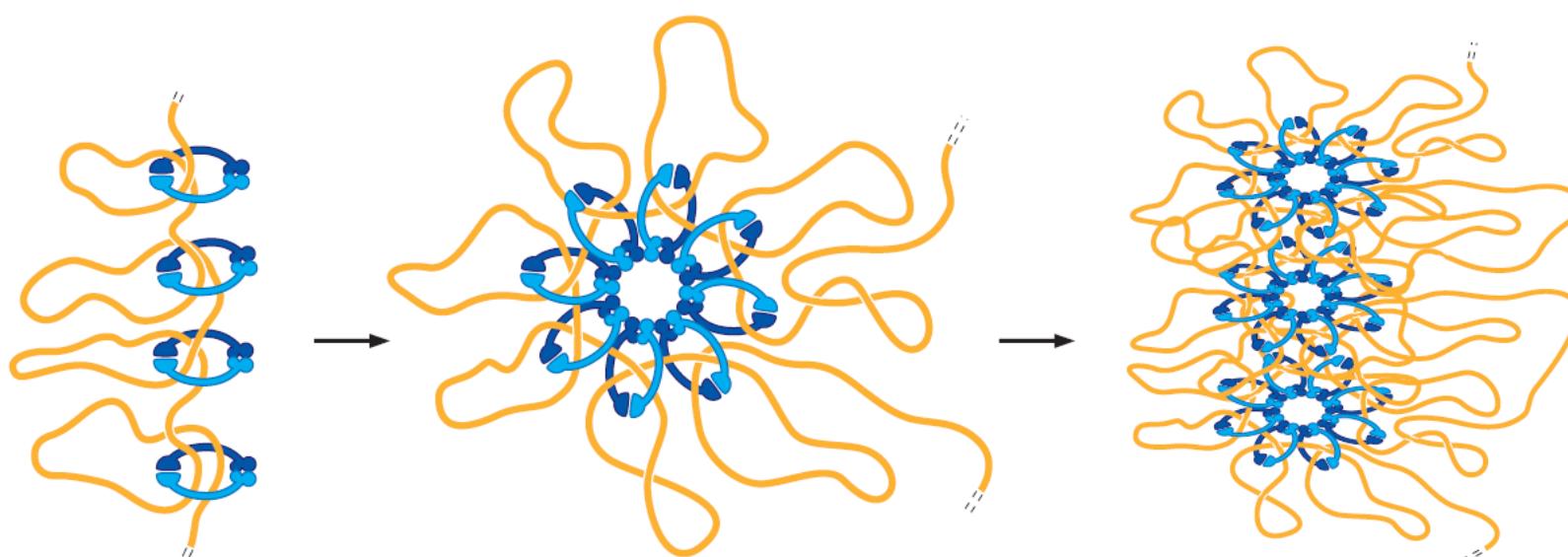
- M-Cdk phosphorylates and activates Cdc25 phosphatase



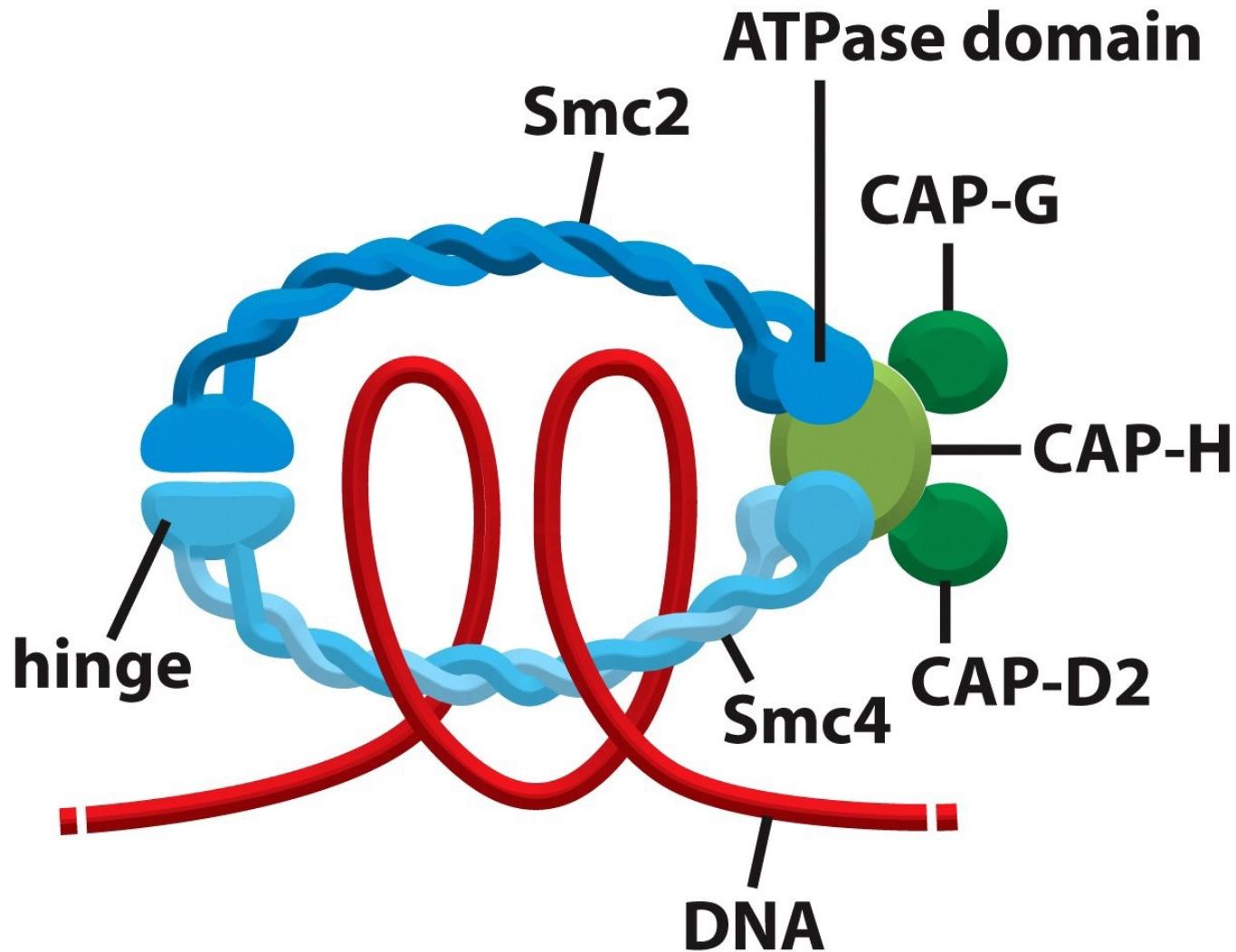
M PHASE: CONDENSINS

➤ Condensins participate in chromosome condensation:

- M-Cdk phosphorylates condensin subunits
- assembly of condensin subunits
- similar to cohesines but are assembled on a single chromatid

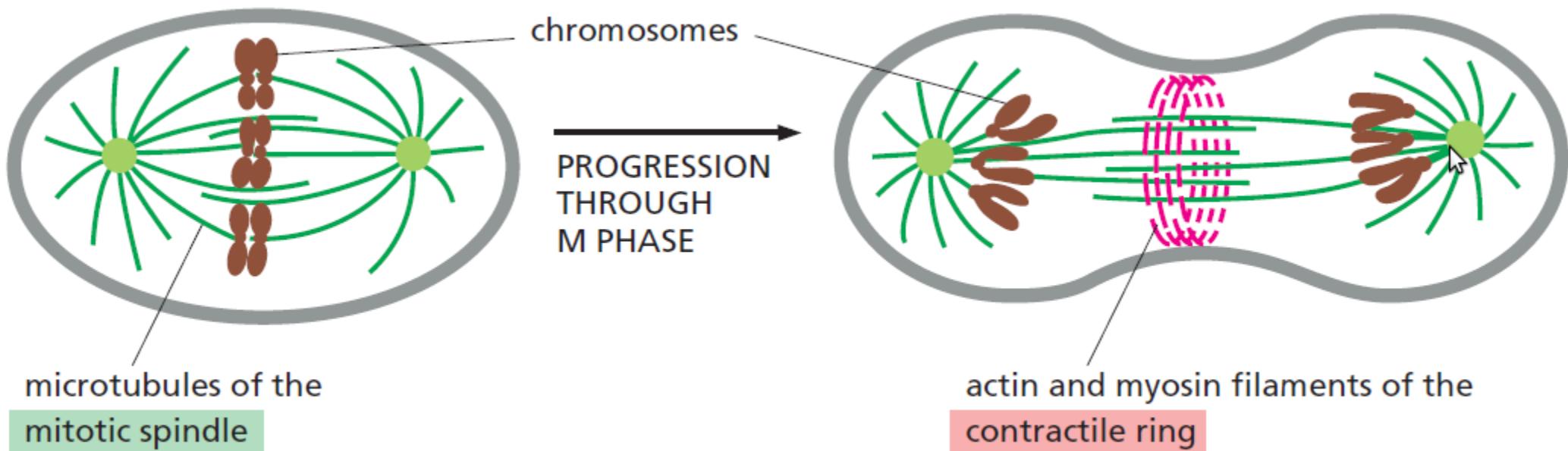


M PHASE: CONDENSINS



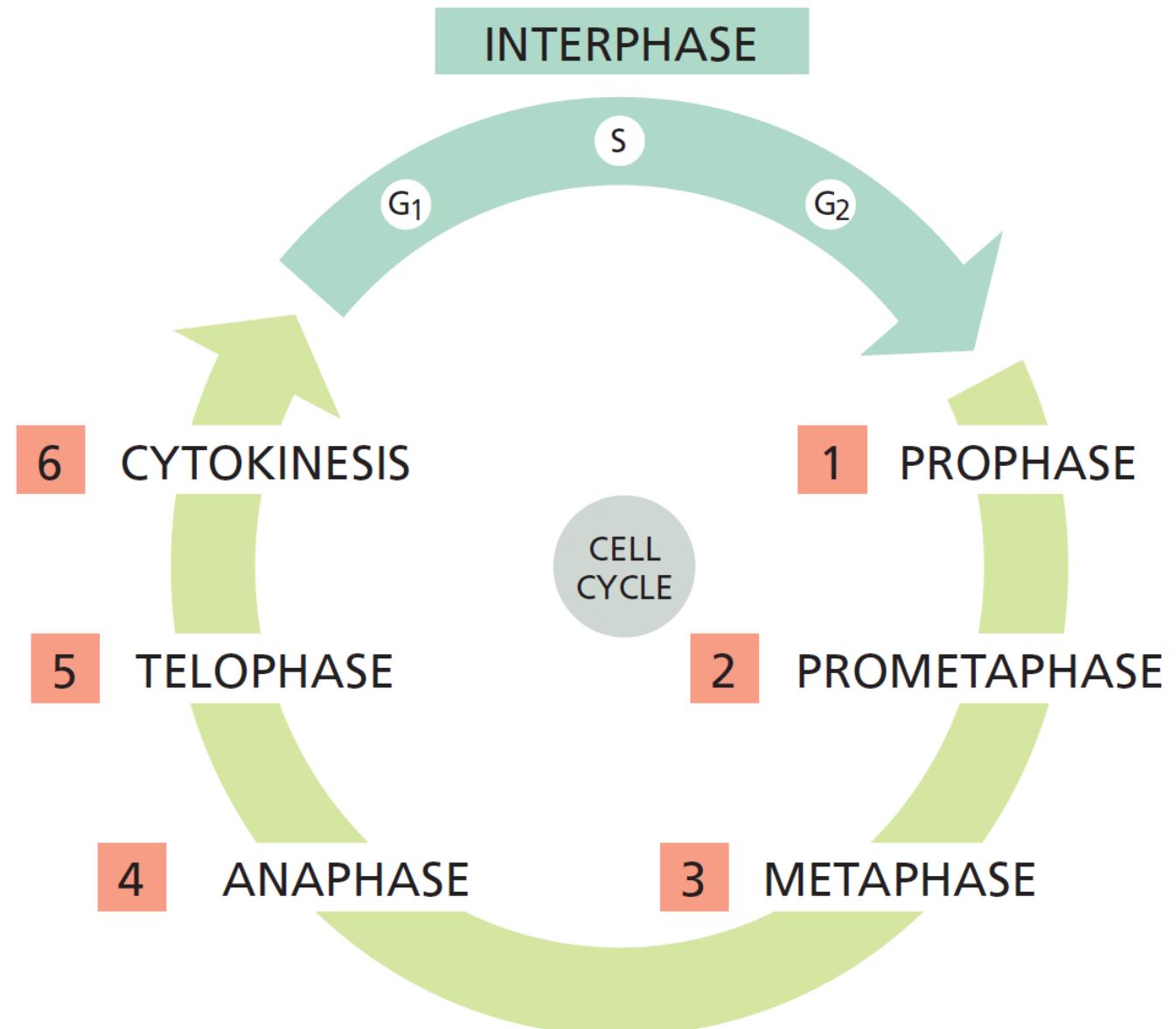
M PHASE: ROLE OF CYTOSKELETON

- Mitotic spindle: nuclear division (mitosis):
 - microtubules + proteins (including motors)
- Contractile ring: cytoplasmic division (cytokinesis)
 - microfilaments (actin) + myosin



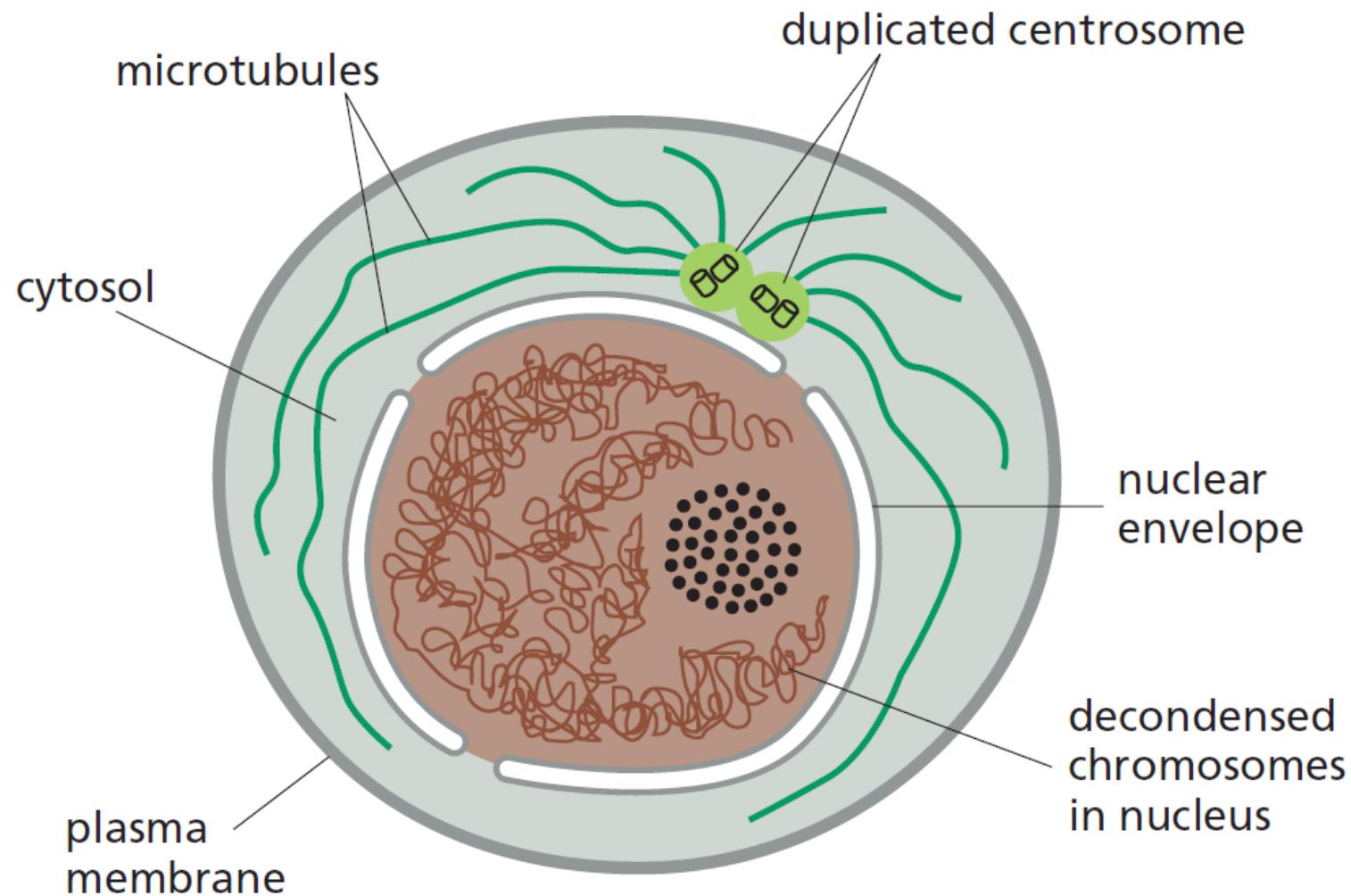
M PHASE STAGES

- Mitosis: 1-5
- Cytokinesis: 4-6



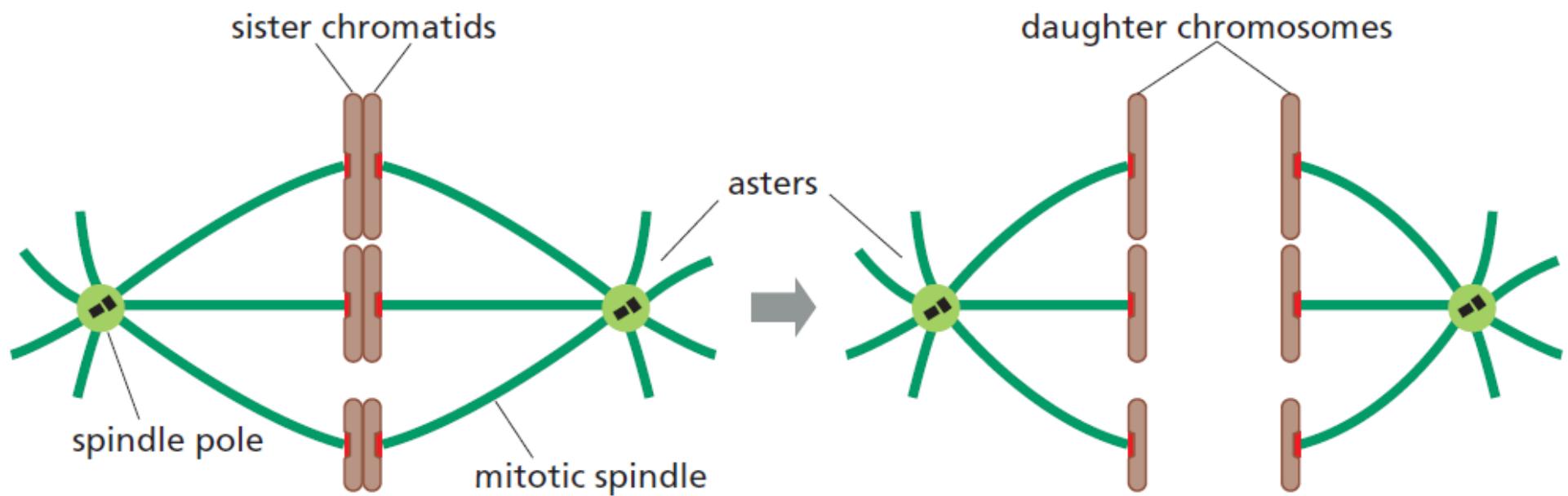
INTERPHASE

- Cell increases in size
- DNA of chromosomes are replicated
- Centrosome is duplicated



BEFORE THE M PHASE

- DNA should be fully replicated
- The centrosome (microtubule organizing center) should be duplicated



CENTROSOME CYCLE

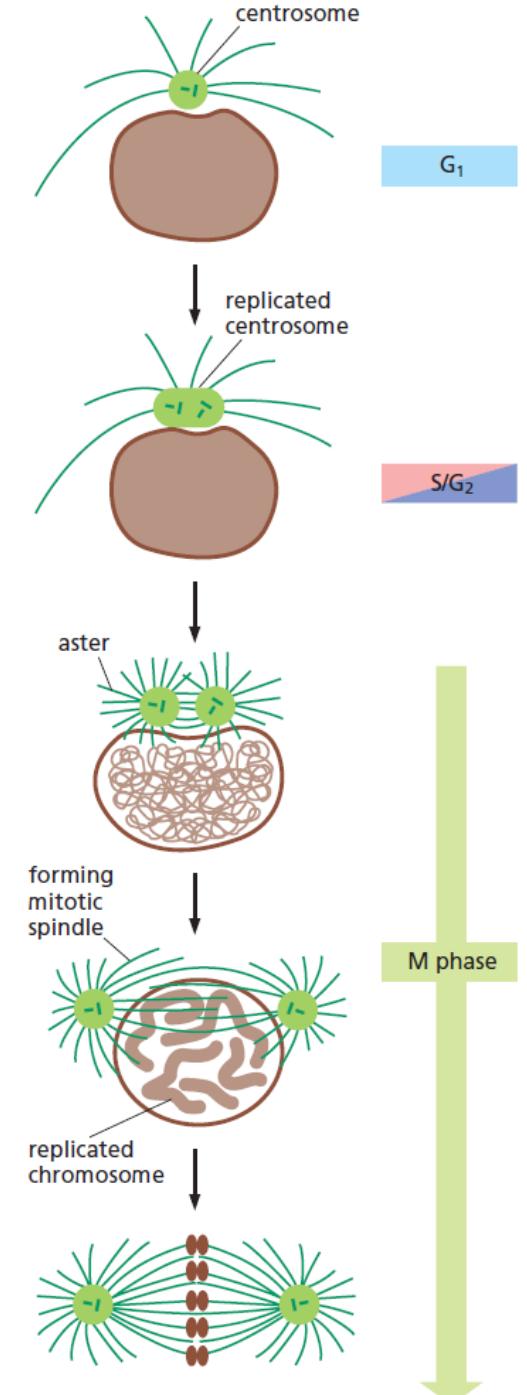
➤ In S phase:

- G1/S-Cdk

- S-Cdk

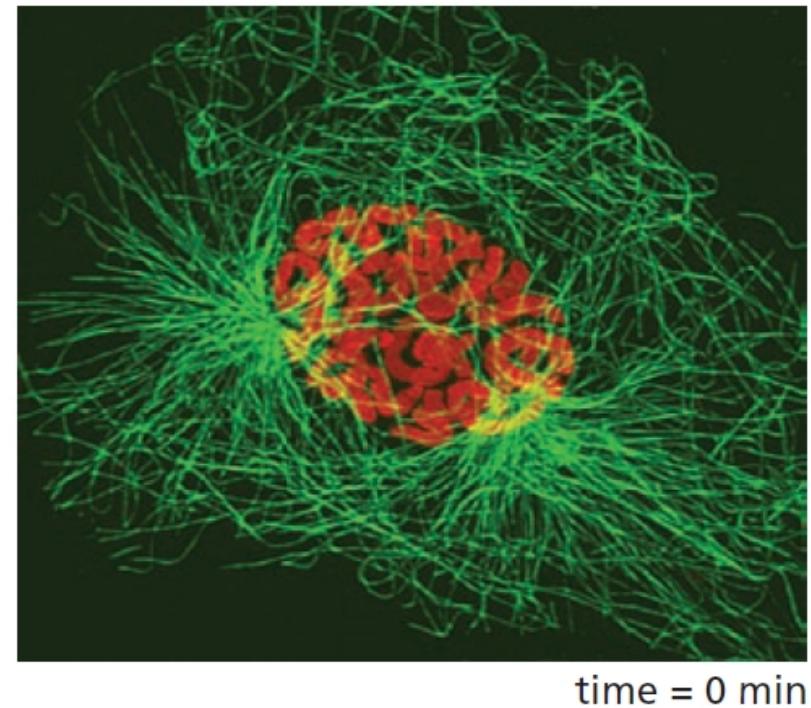
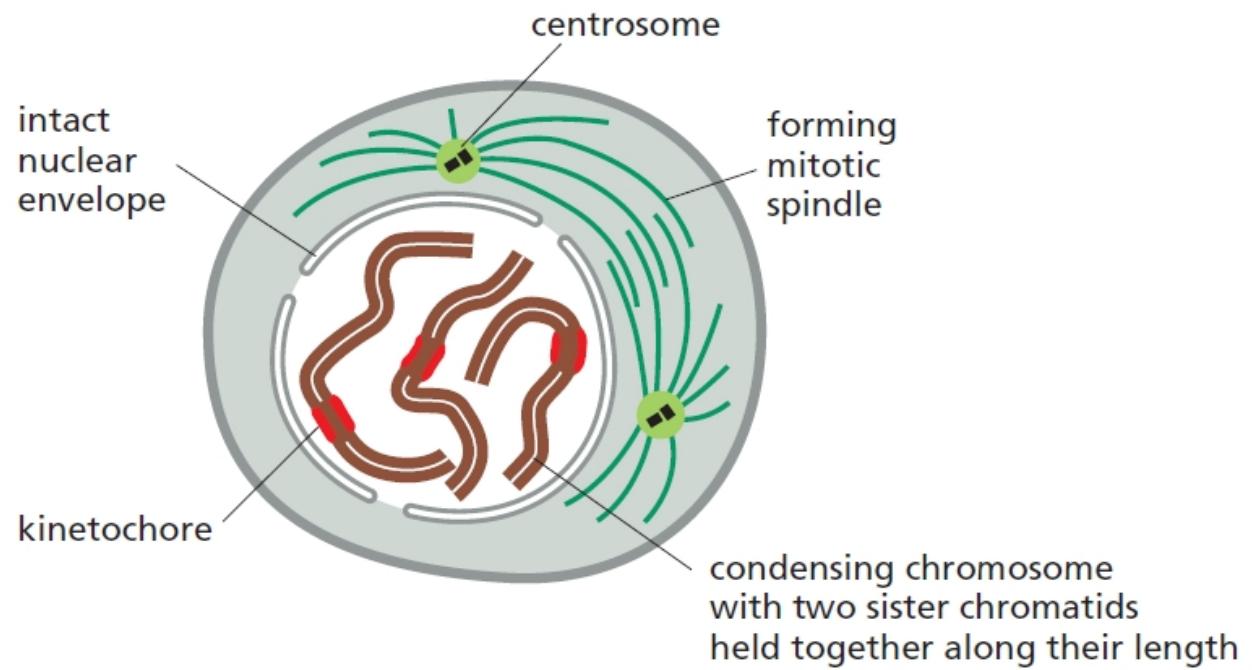
➤ Aster: nucleated new radial array of microtubules

➤ Two spindles formation



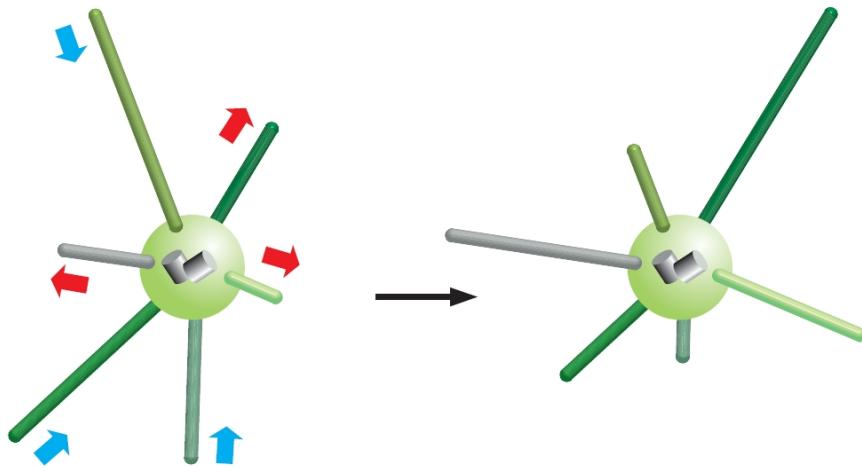
M PHASE STAGES: 1. PROPHASE

- Replicated chromosomes condense
- Mitotic spindle assembles between the two centrosomes

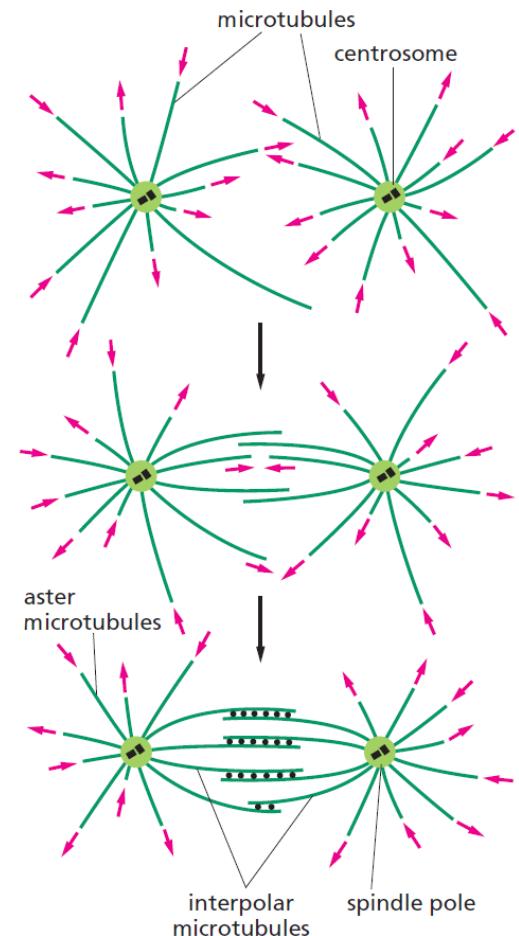


M PHASE STAGES: 1. PROPHASE

- Increase of dynamic instability of microtubules
- Microtubules grow/shrink rapidly and explore the interior of the cell
- Some of them interact with centrosomes and are so stabilized
- Formation of mitotic spindles, spindle poles, interpolar microtubules
- Motor proteins association
- Spindle microtubules attach to kinetochores

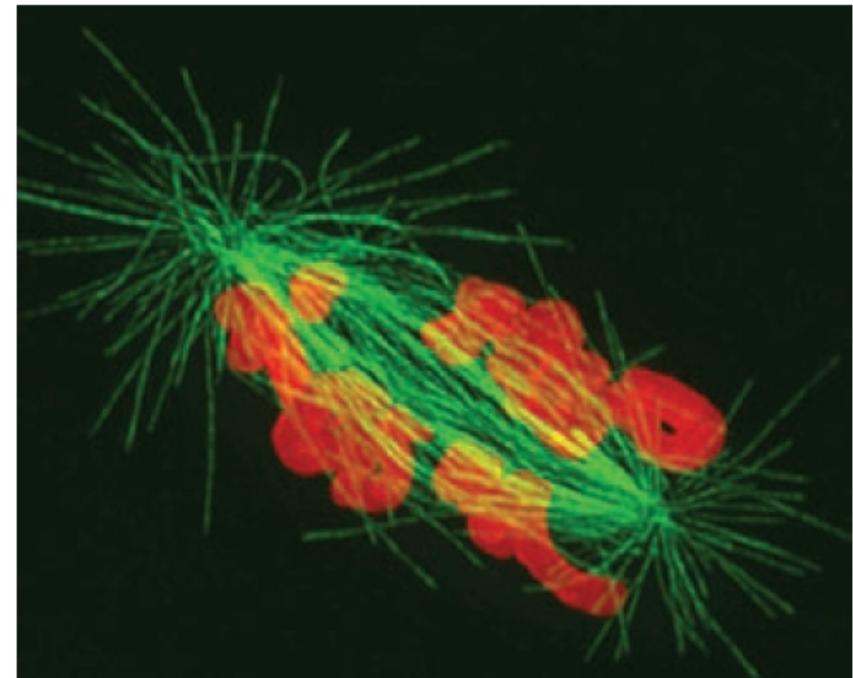
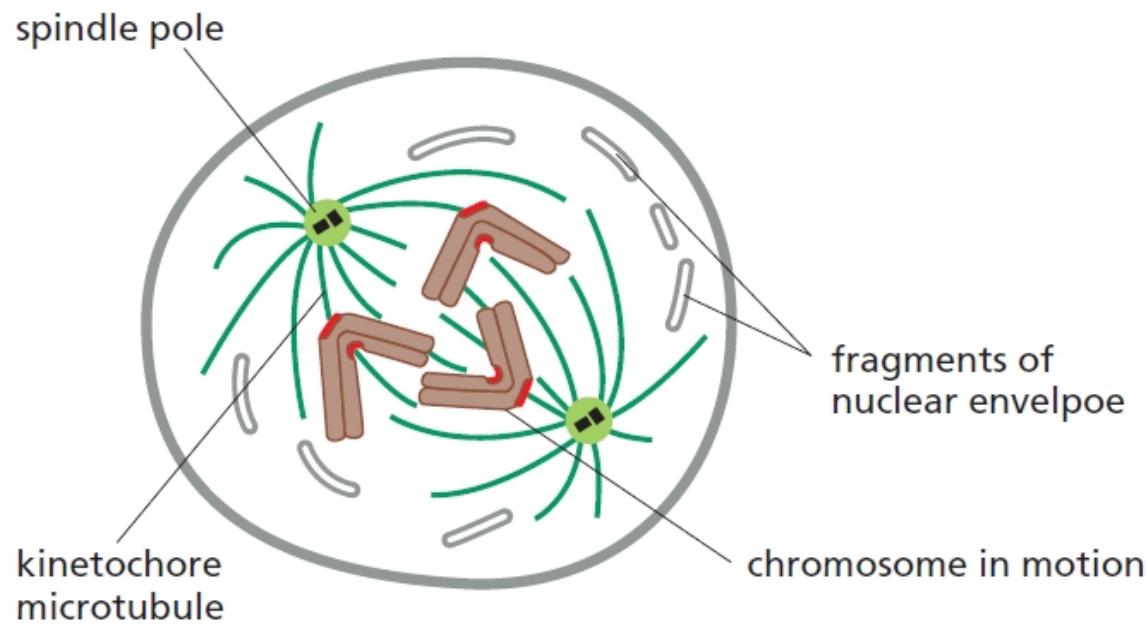


Dynamic instability of microtubules



M PHASE STAGES: 2. PROMETAPHASE

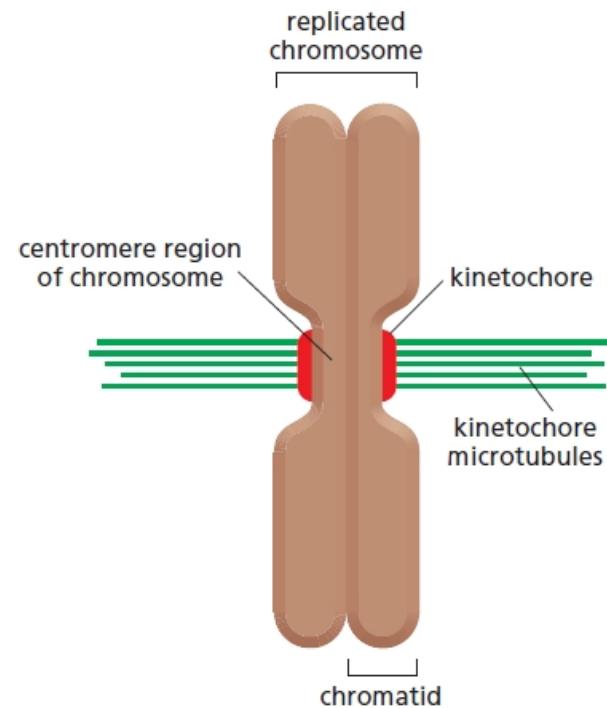
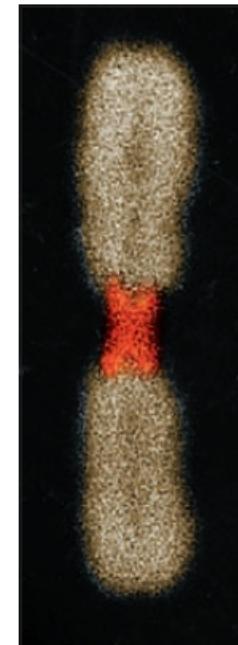
- Breakdown of nuclear envelope
- Chromosomes attach to spindle microtubules via kinetochores



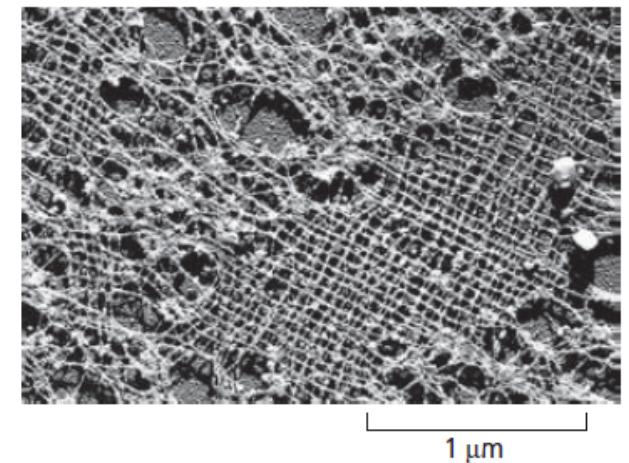
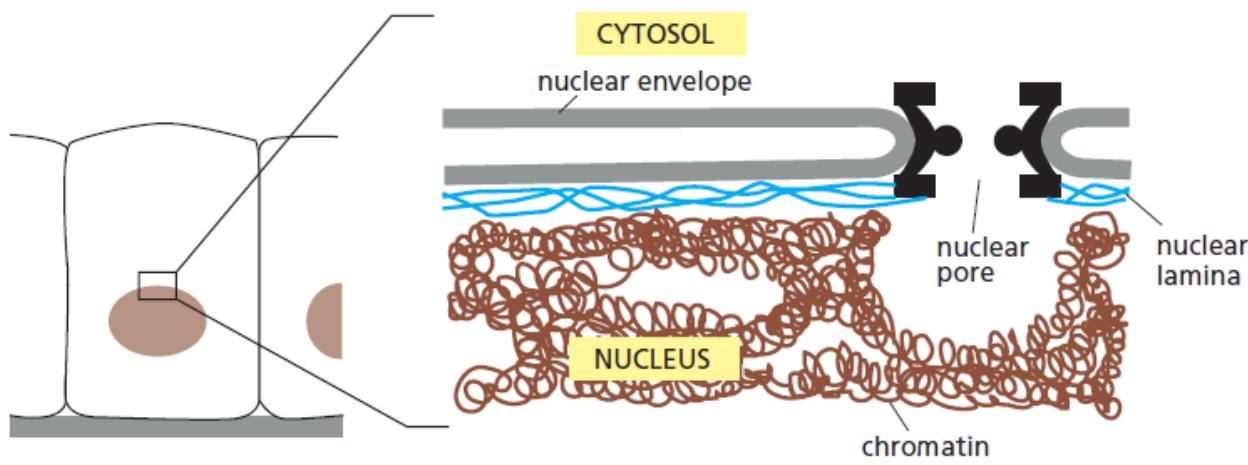
time = 79 min

M PHASE STAGES: 2. PROMETAPHASE

- Nuclear envelope => vesicles, triggered by phosphorylation:
 - nuclear pore proteins
 - intermediate filaments of nuclear lamina
 - fibrous proteins underlying nuclear envelope

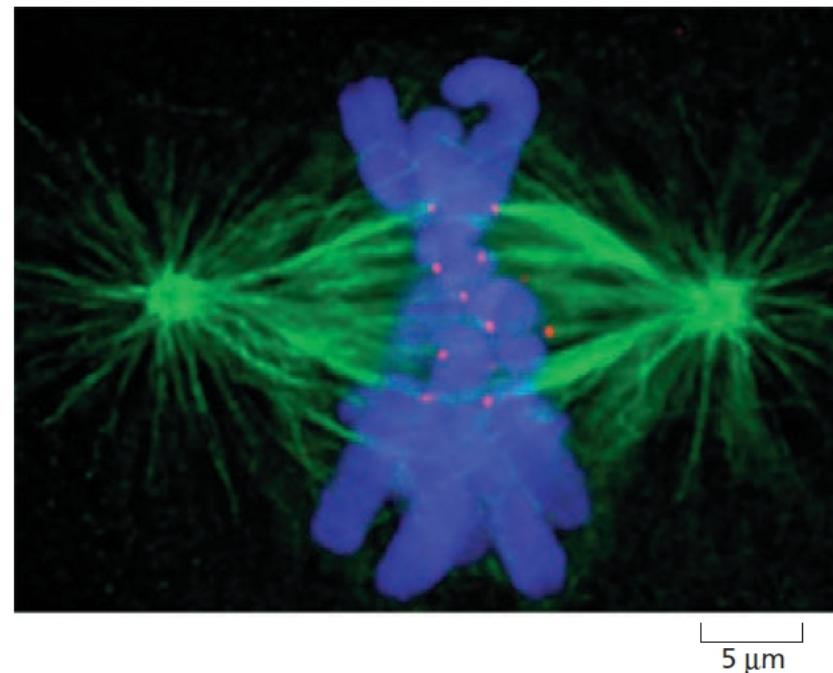
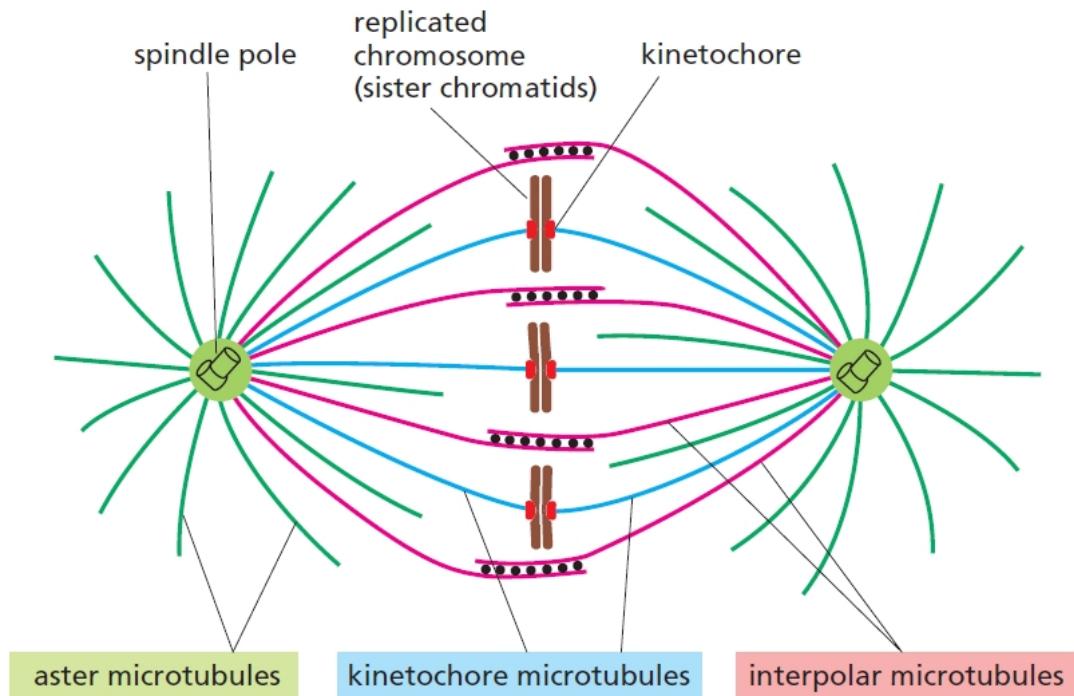


- Kinetochore proteins assemble into a large complex on centromere

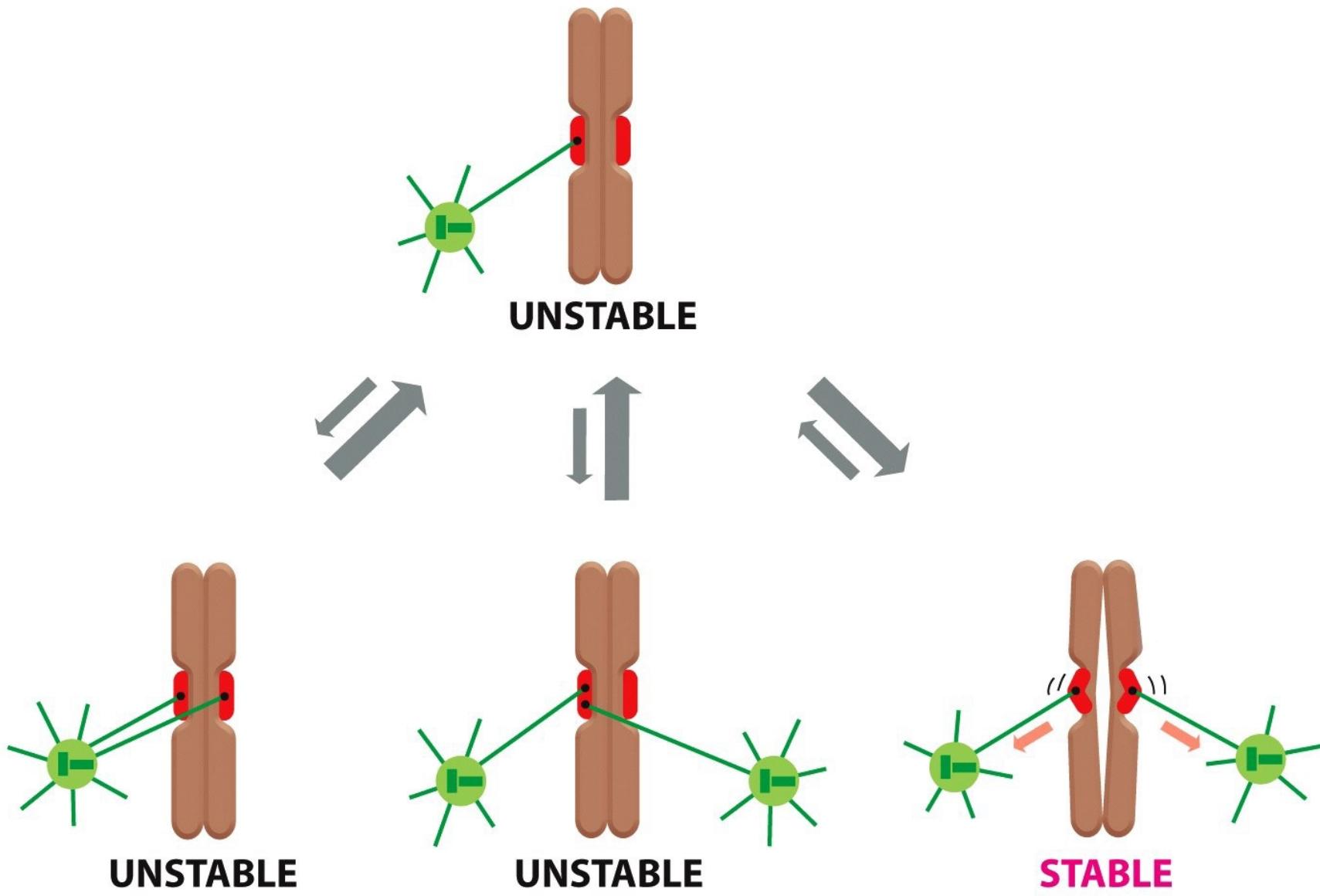


M PHASE STAGES: 2. PROMETAPHASE

- Kinetochores and spindle => polarity, bi-orientation
- => tension to be pulled in opposite directions
- Different classes of microtubules

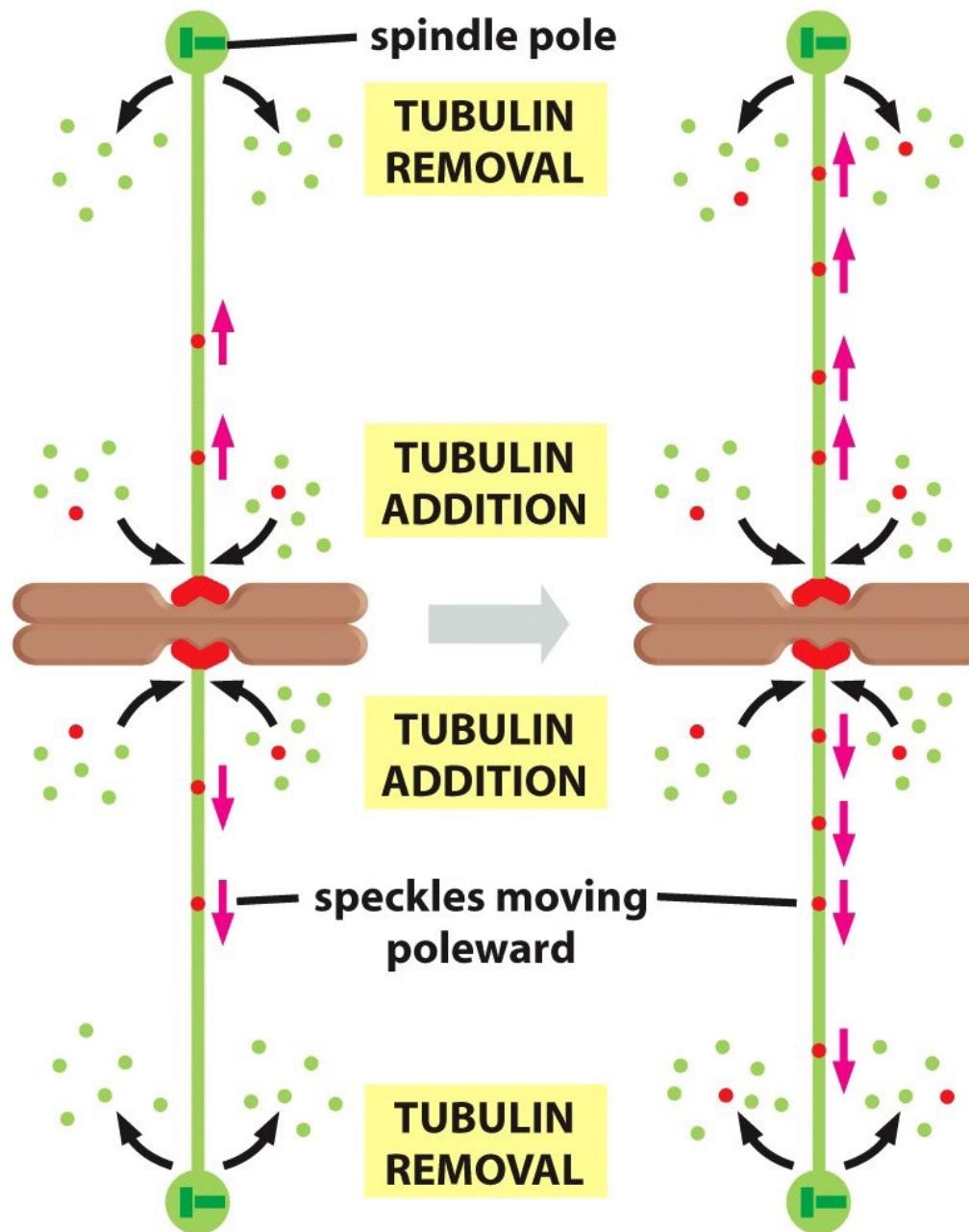


M PHASE STAGES: 2. PROMETAPHASE



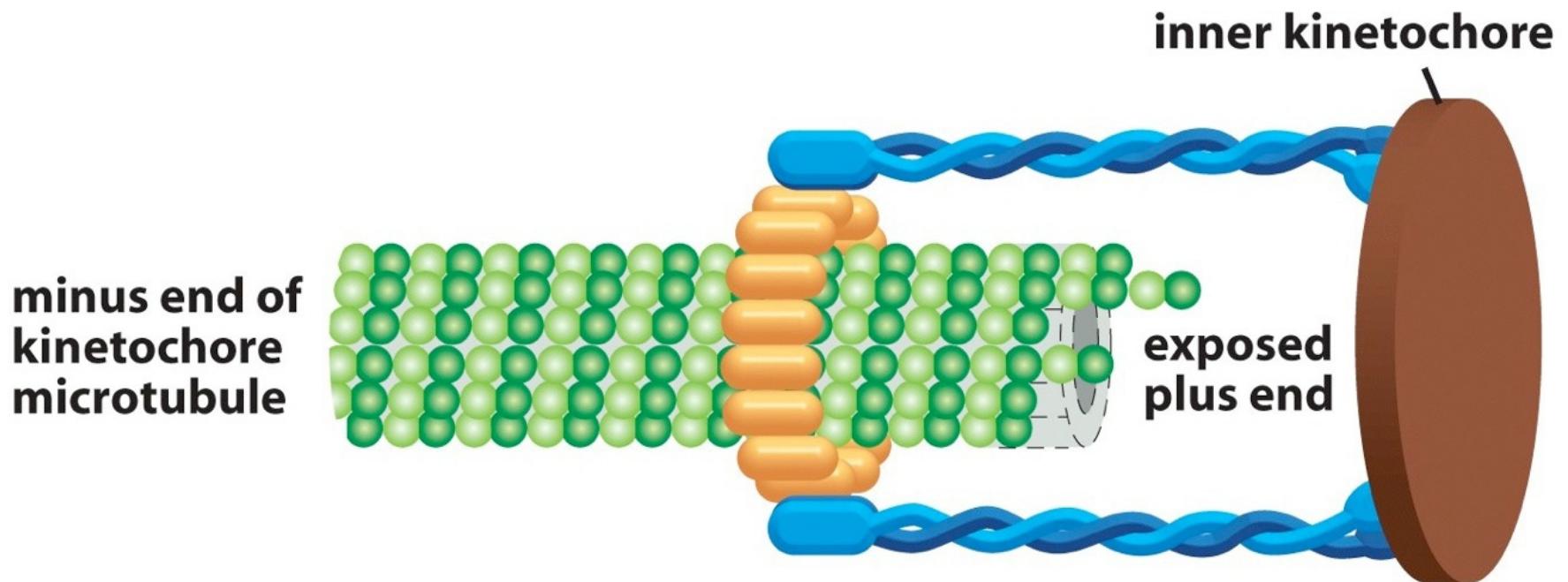
Bi-orientation is achieved by 'try and error'

M PHASE STAGES: 2. PROMETAPHASE



Highly dynamic structure: tubulin exchange

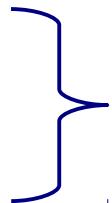
M PHASE STAGES: 2. PROMETAPHASE



M PHASE STAGES: 2. PROMETAPHASE

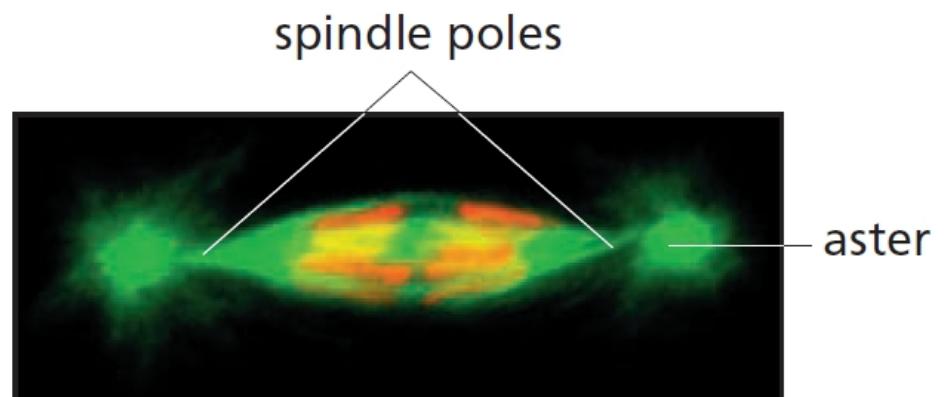
➤ No centrosomes:

- chromosomes
- motor proteins

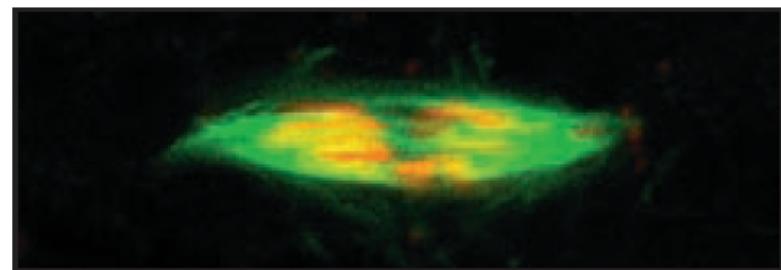


arrangement in bipolar spindles

with centrosomes



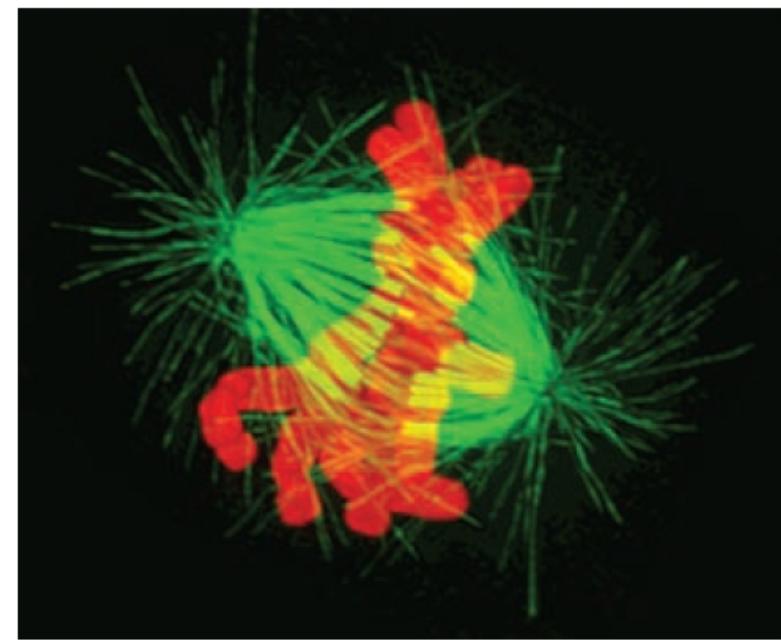
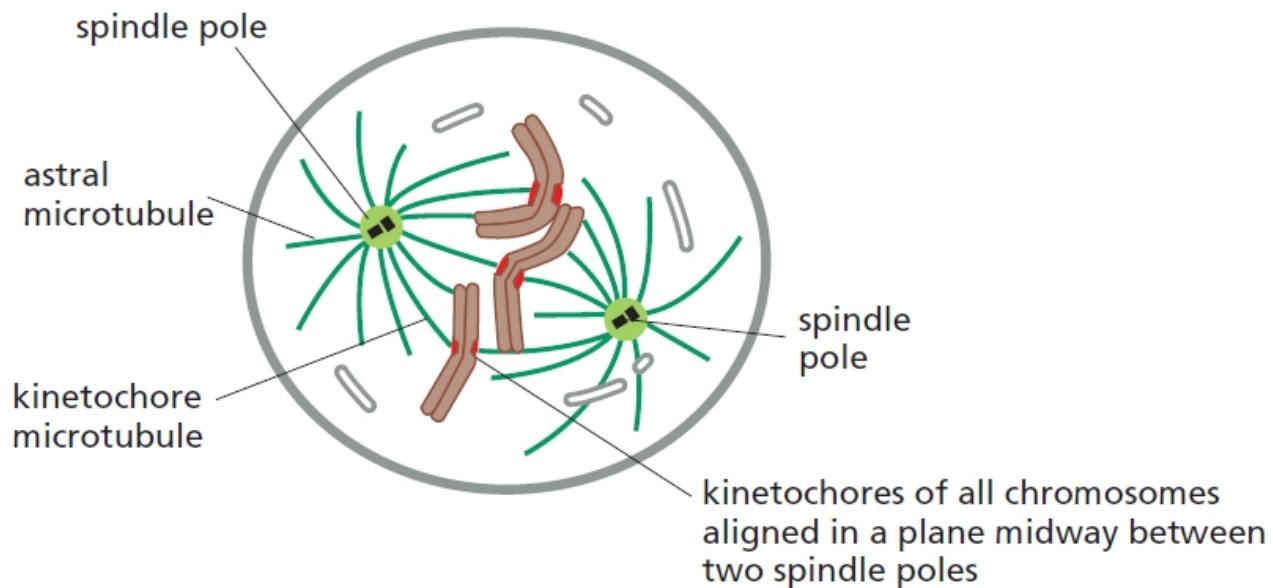
without centrosomes



10 μm

M PHASE STAGES: 3. METAPHASE

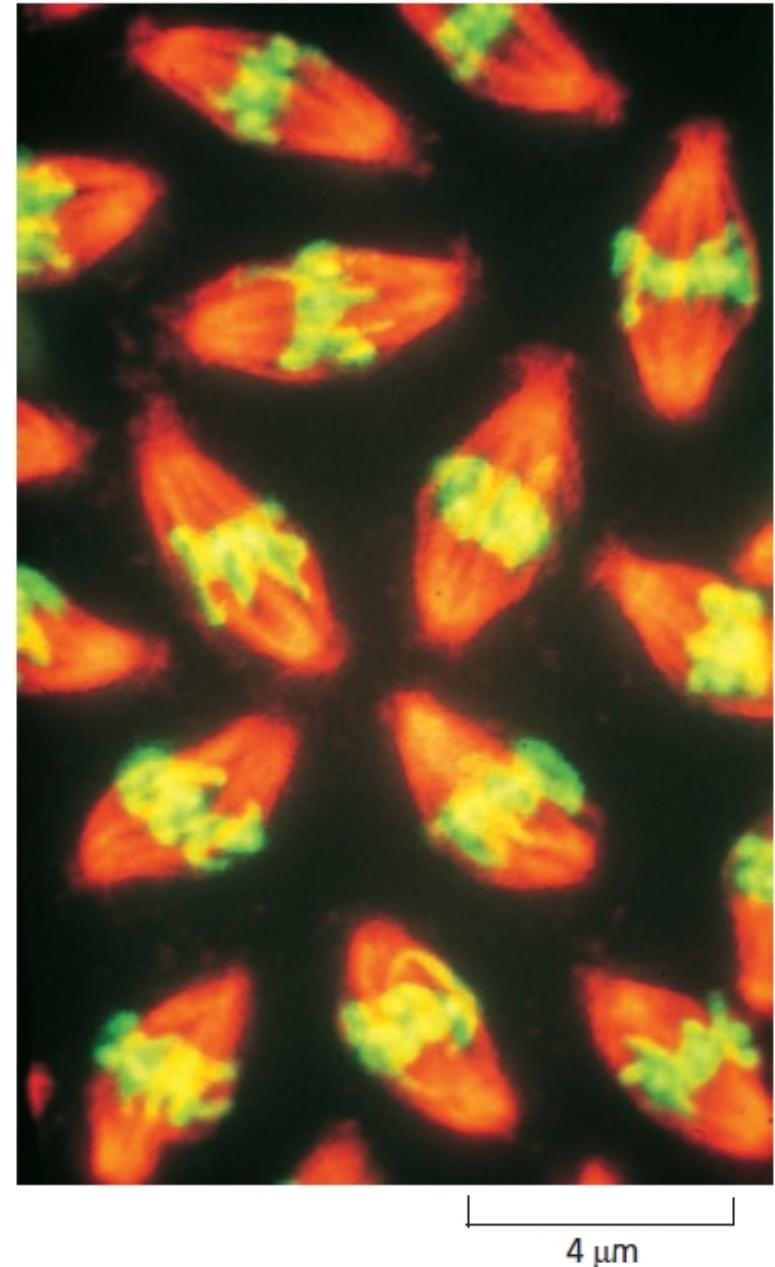
- Chromosomes are aligned at the equator of the spindle in the middle
- The paired kinetochore microtubules on each chromosome attach to opposite poles of the spindle



time = 250 min

M PHASE STAGES: 3. METAPHASE

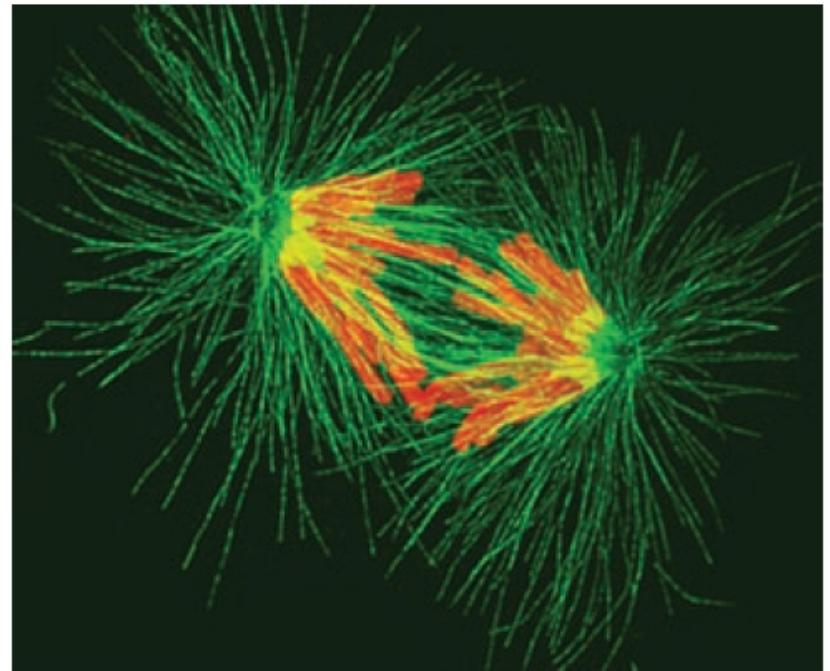
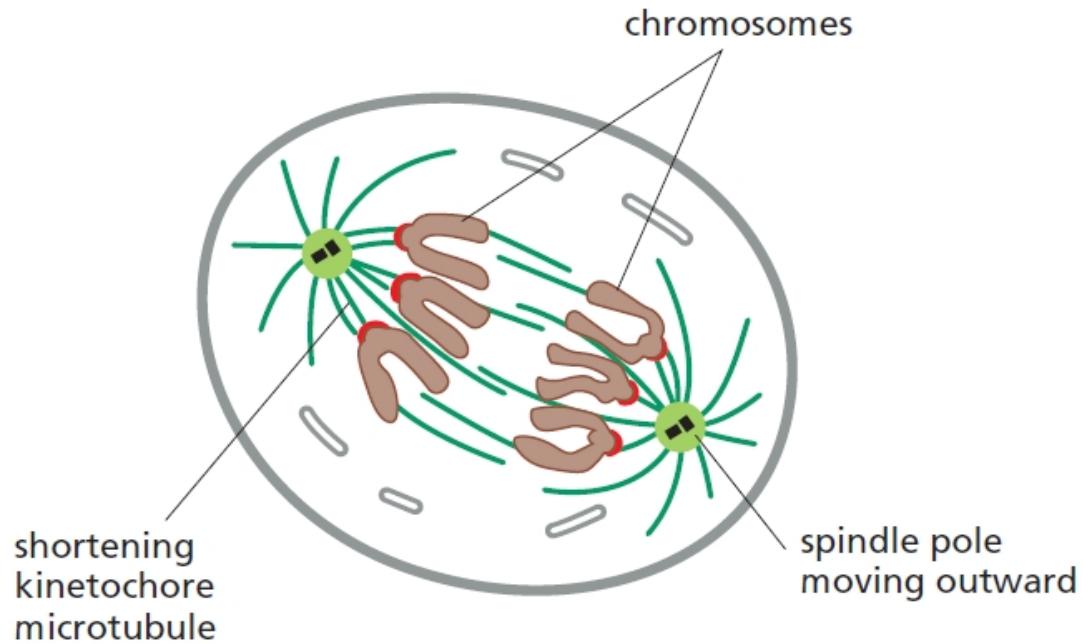
- Chromosomes in a half-way of the equator of the spindle: metaphase plate
- Involvement of dynamic instabilities of microtubules
- Colchicine: spindle disappears
- Chromosomes are held by tension (an experiment with laser cut)



Drosophila metaphase:
cells are synchronized

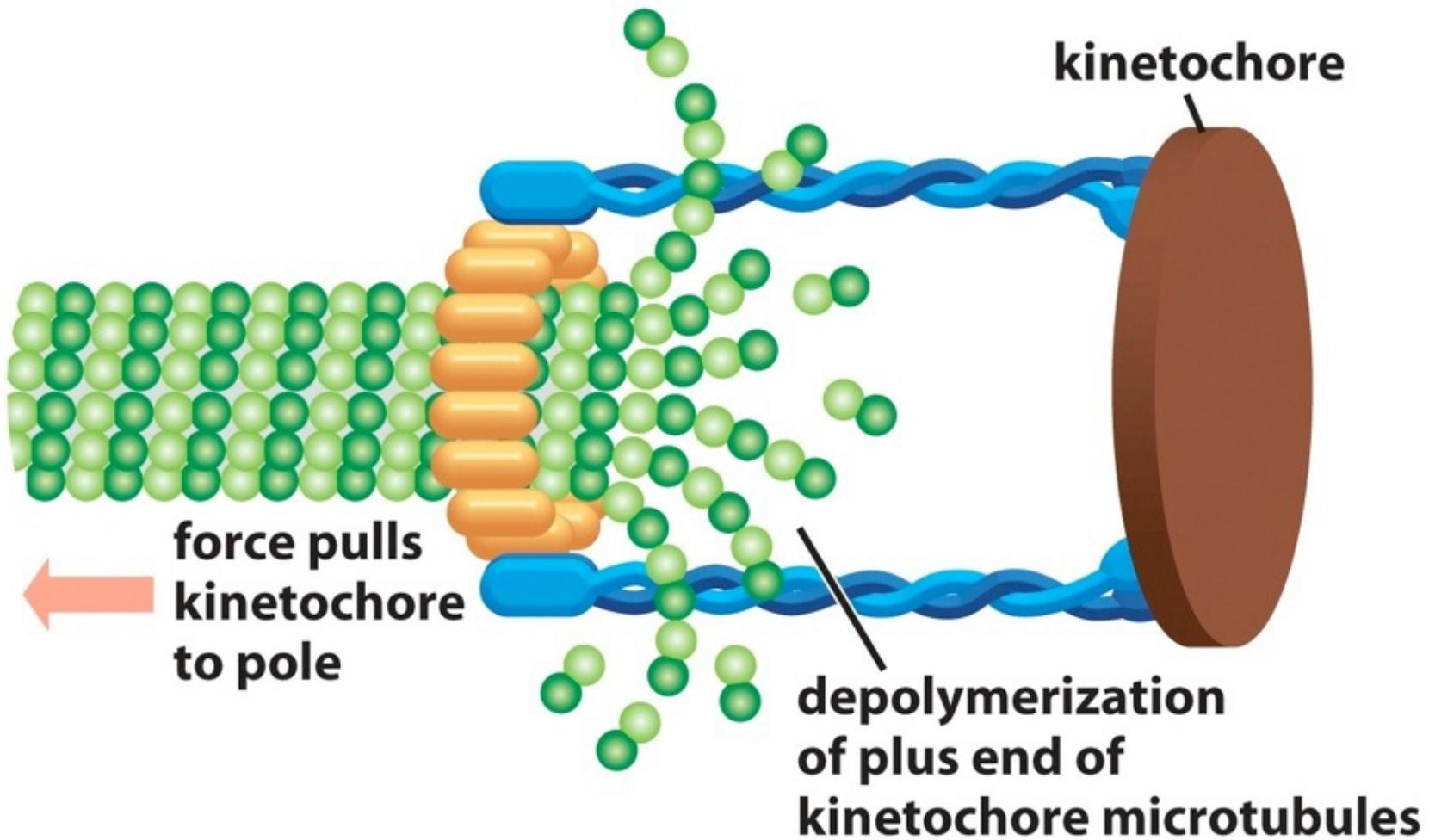
M PHASE STAGES: 4. ANAPHASE

- Spindle assembly checkpoint: 'stop signal' from unattached chromosomes
- Sister chromatids synchronously separate
- Each one is pulled toward own spindle pole
- Kinetochore microtubules are shortened, spindles move apart



time = 279 min

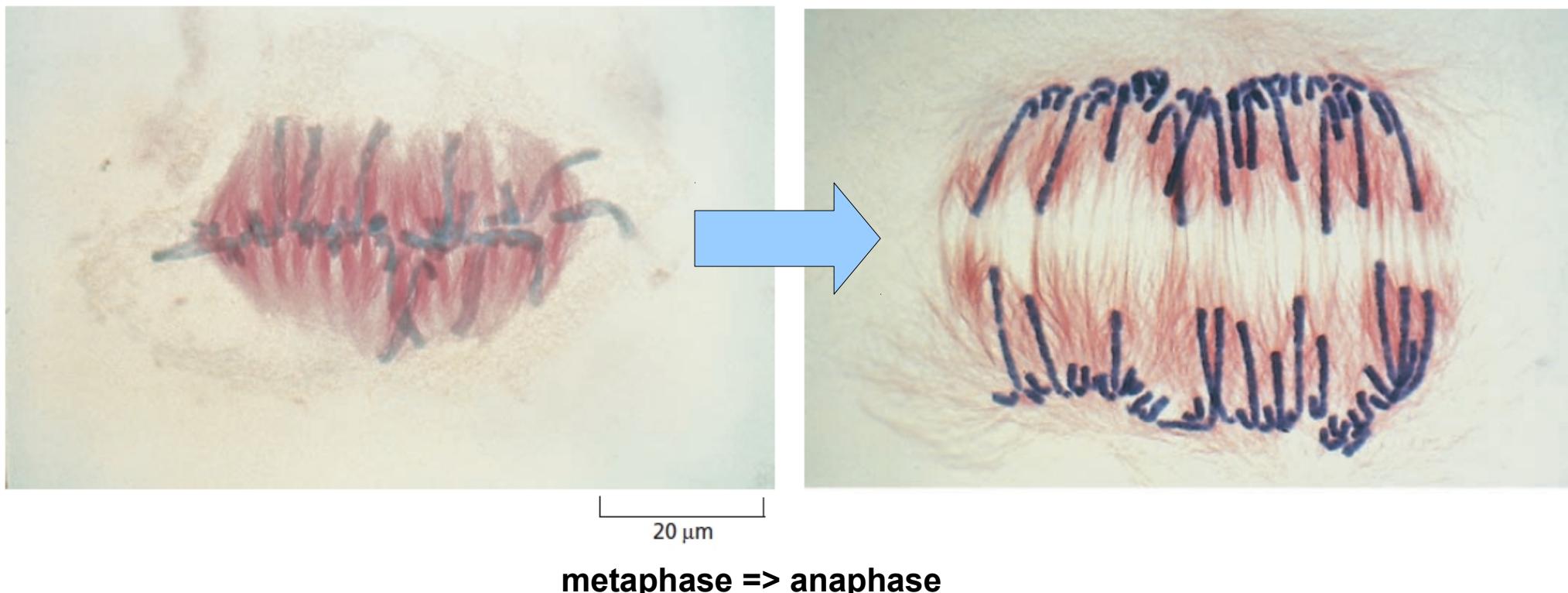
M PHASE STAGES: 4. ANAPHASE



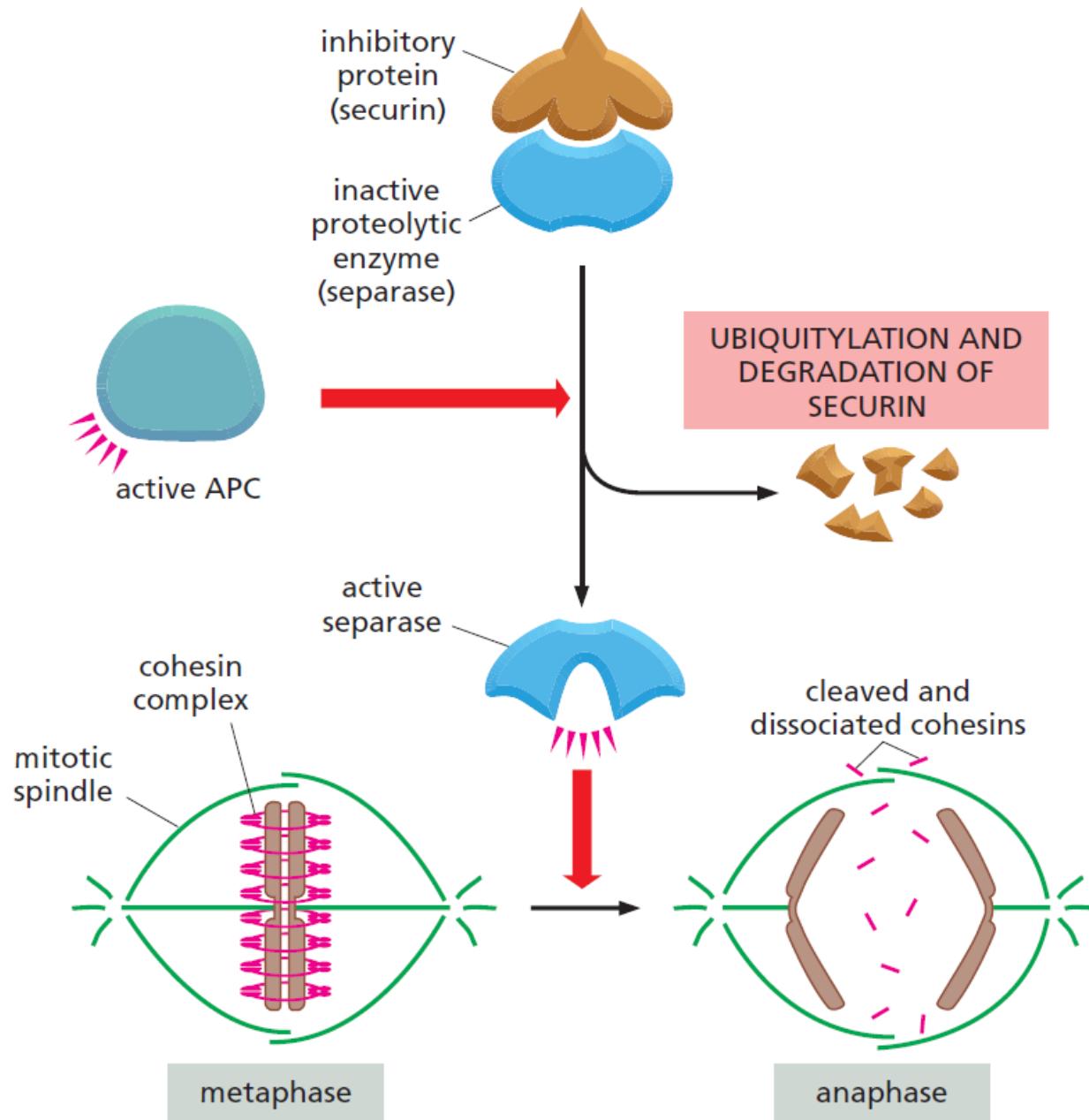
Catastrophin uses ATP-energy to depolymeraze microtubules

M PHASE STAGES: 4. ANAPHASE

- Release of cohesin linkage => movements to the opposite ends of the spindle (~1µm/min)
- Cohesin is destroyed by protease separase
- Securin is an inhibitor of separase
- Anaphase promoting complex (APC) destroys securin (L23-24)

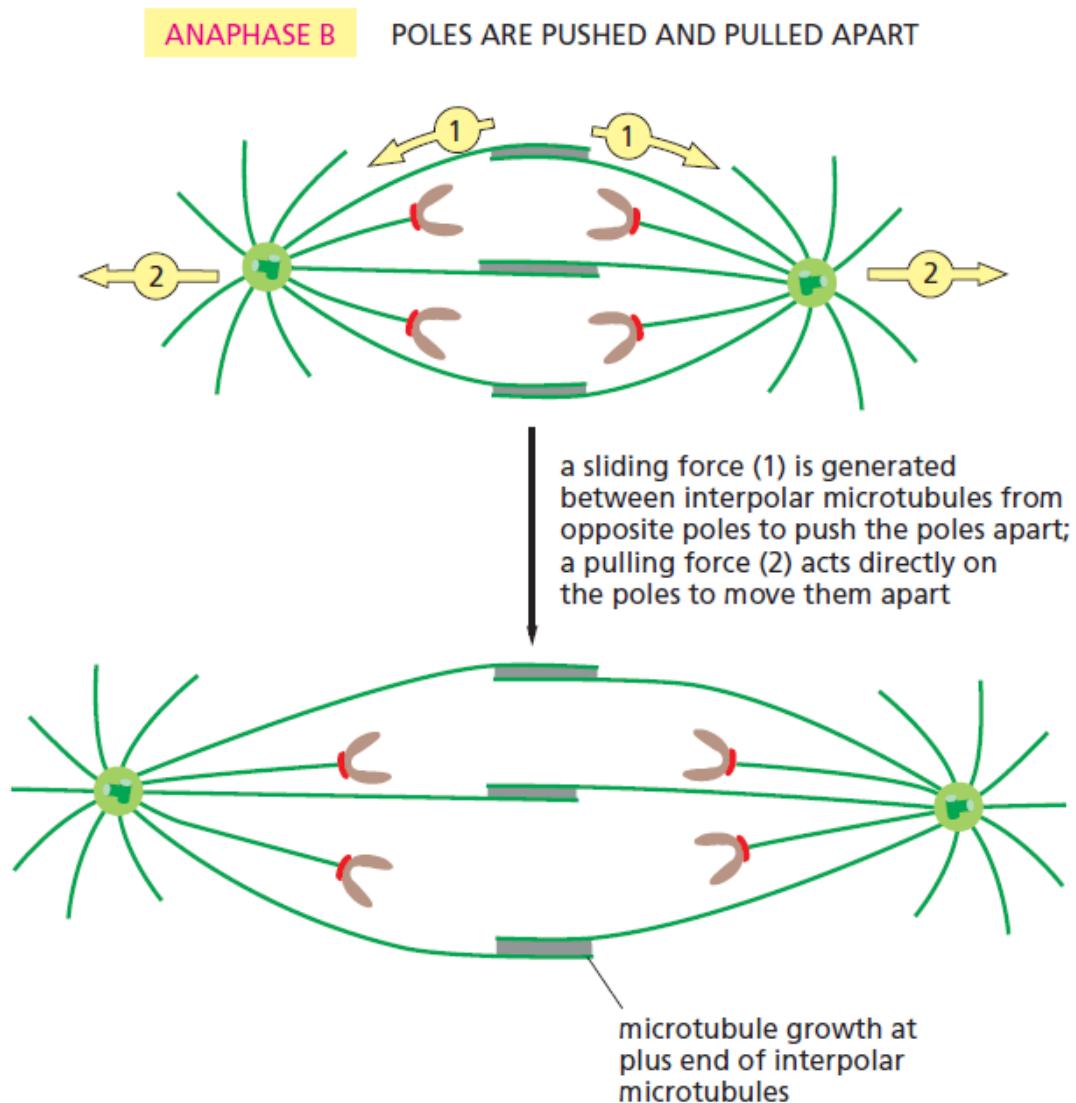
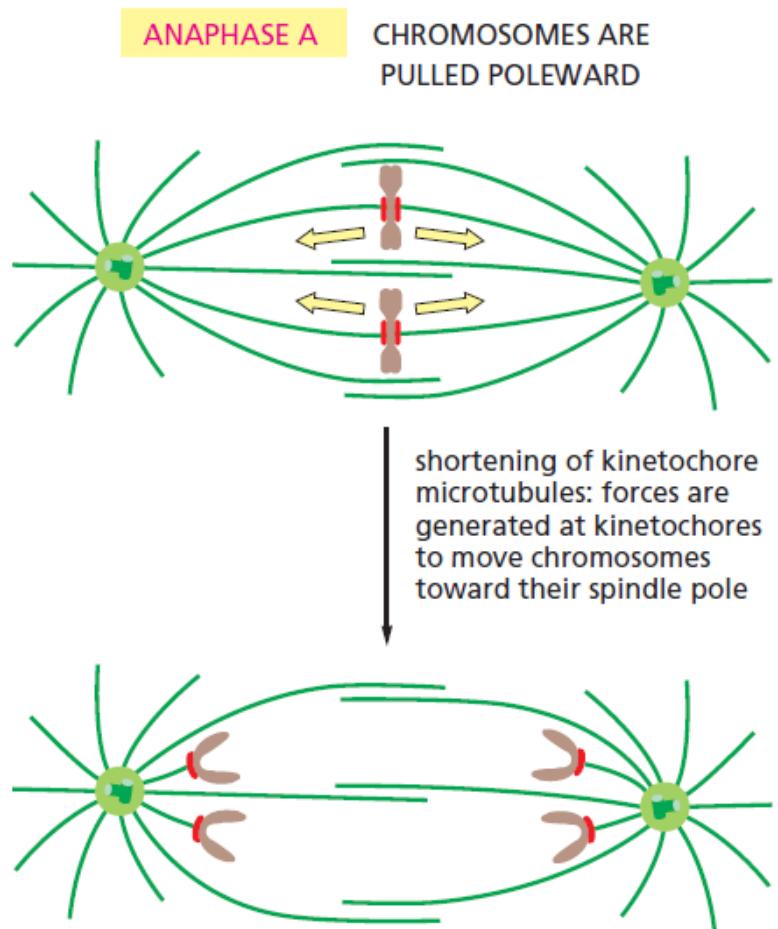


M PHASE STAGES: 4. ANAPHASE



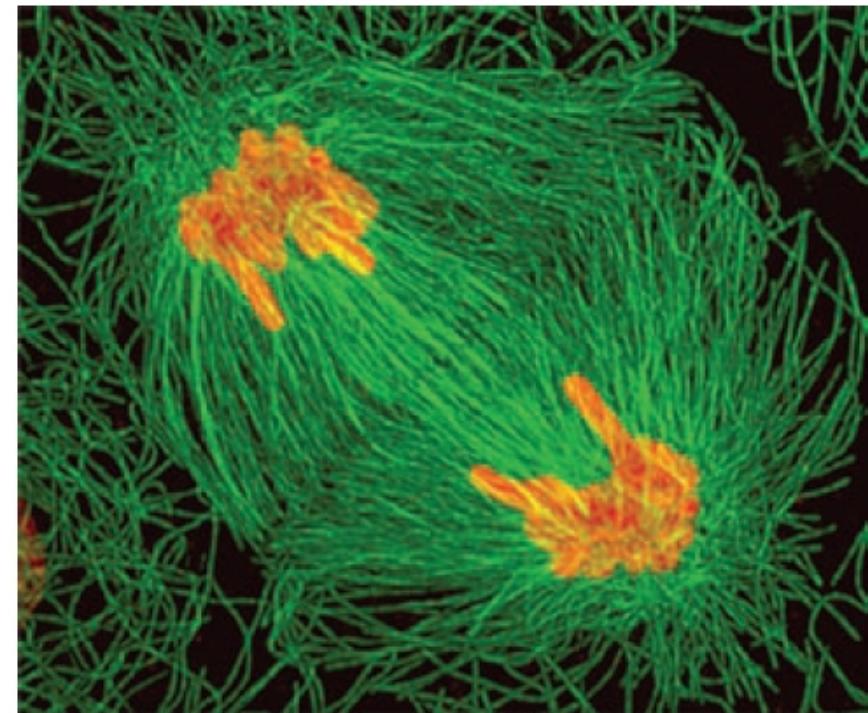
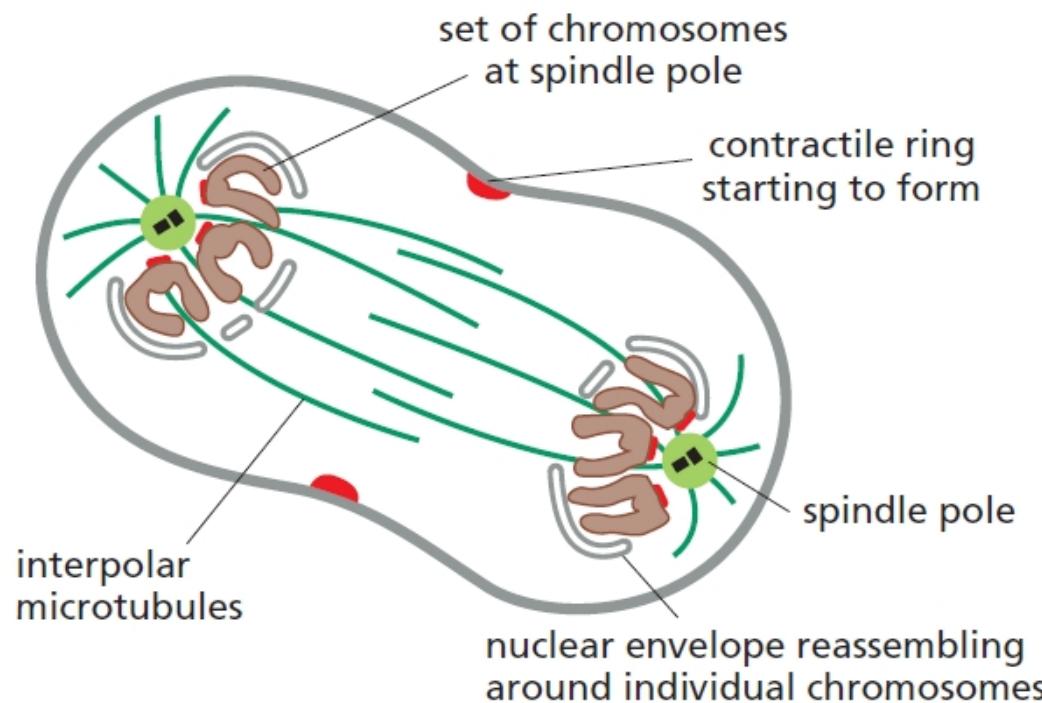
M PHASE STAGES: 4. ANAPHASE

- Anaphase A (microtubules disassembly)
- Anaphase B (motor proteins associated with the cell cortex)



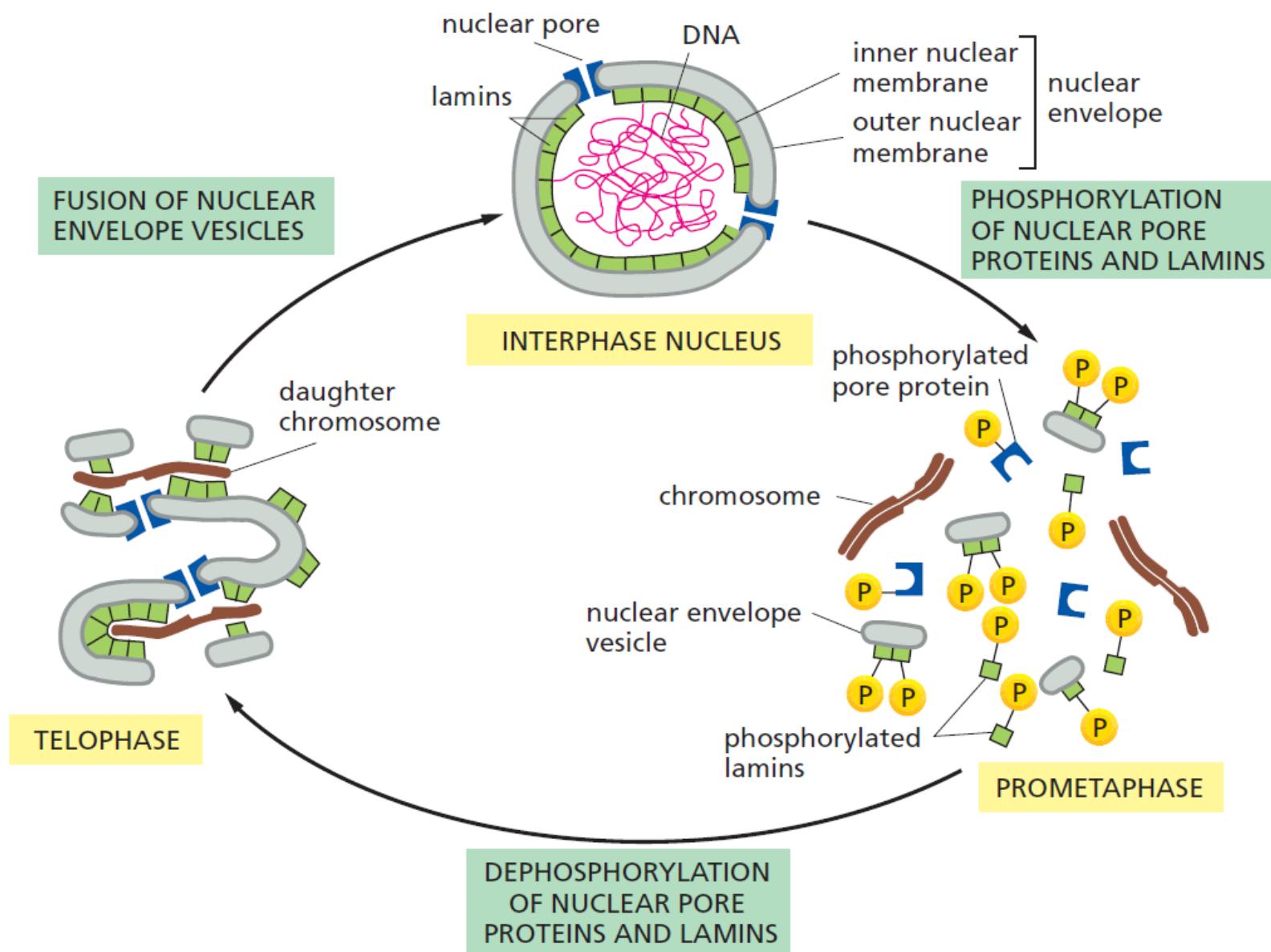
M PHASE STAGES: 5. TELOPHASE

- Two sets of chromosomes arrive at the poles of the spindle
- A new nuclear envelope reassembles => two nuclei => end of mitosis
- Start of cytoplasm division => start of cytokinesis



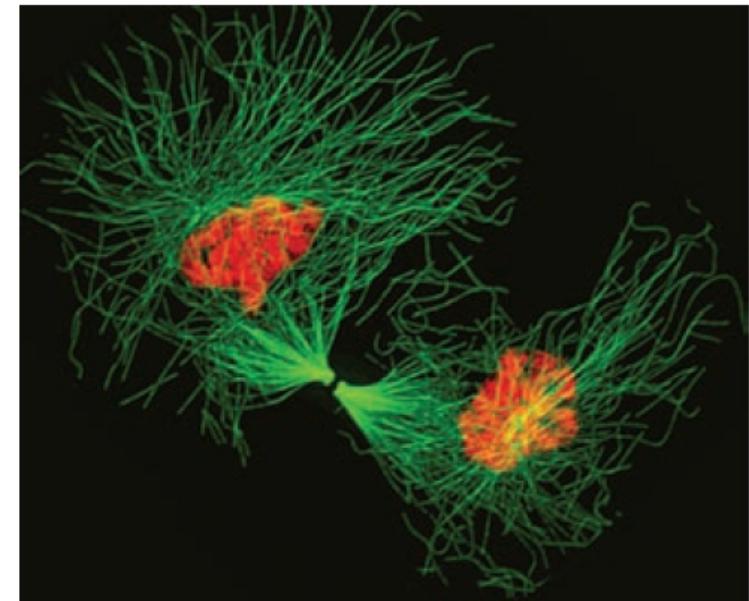
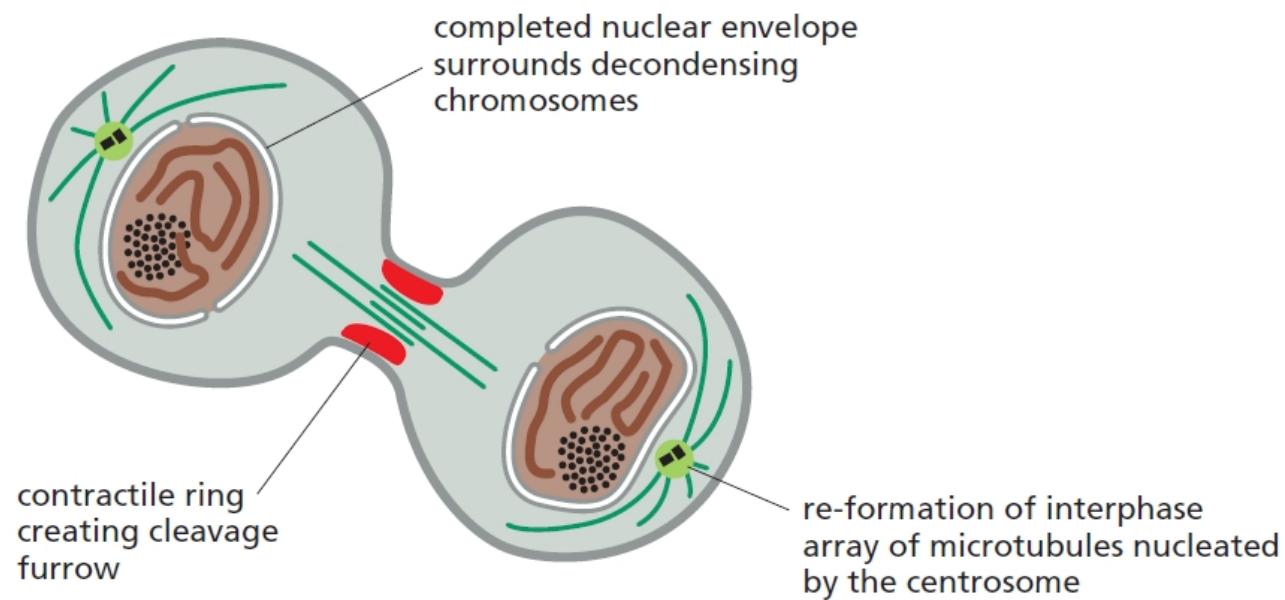
time = 315 min

M PHASE STAGES: 5. TELOPHASE



M PHASE STAGES: CYTOKINESIS

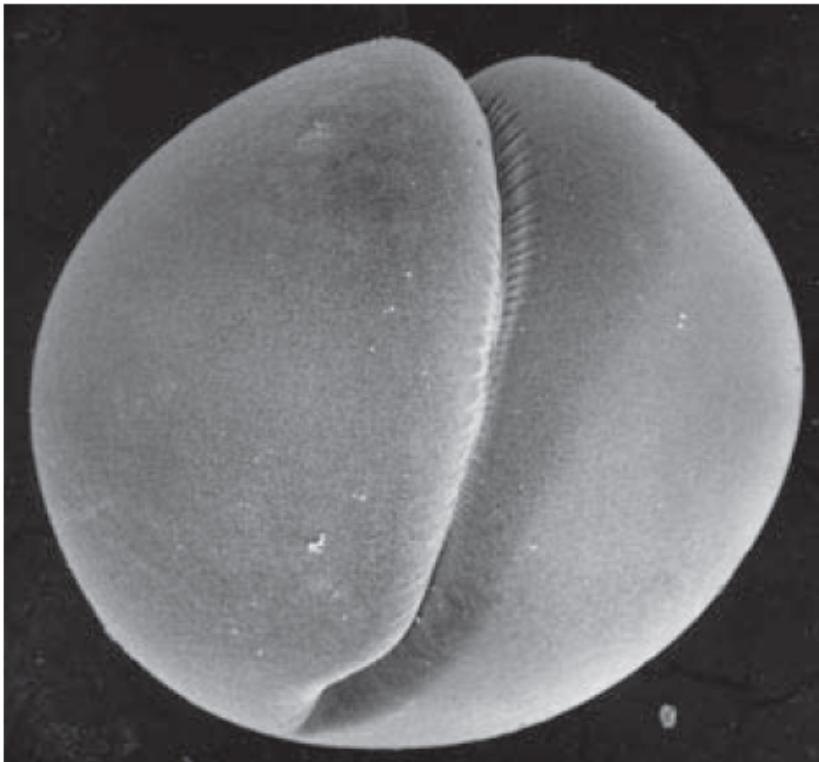
- Cytoplasm is divided in two by a contractile ring of actin/myosin filaments
- Formation of two daughter cells, each with one nucleus



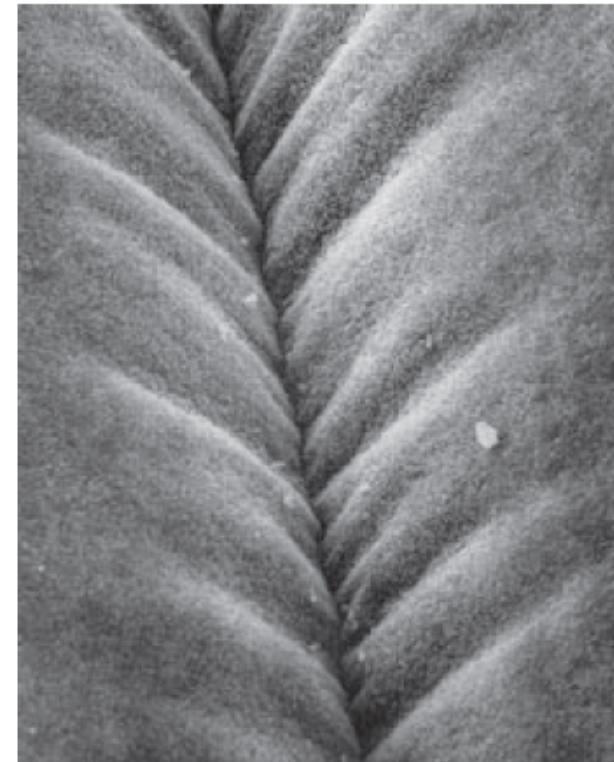
time = 362 min

M PHASE STAGES: CYTOKINESIS

- Cleavage furrow
- Mitotic spindle displacement => furrow disappears => new spindle
- Central mitotic spindle => symmetry => daughter cells of the same length
- Embryonic divisions => assymetric



200 μm

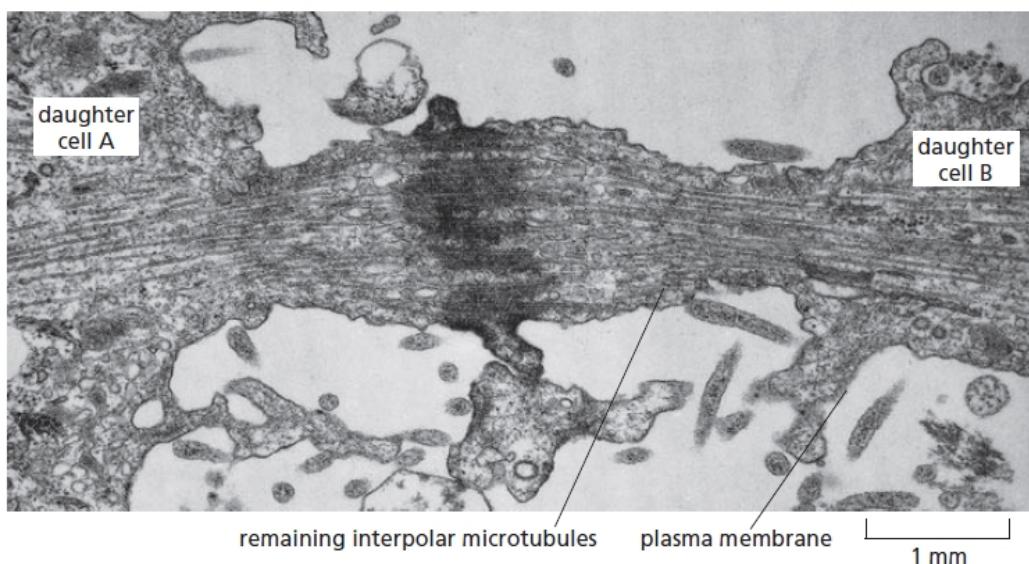
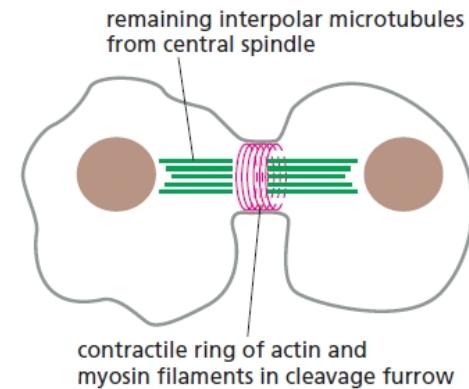
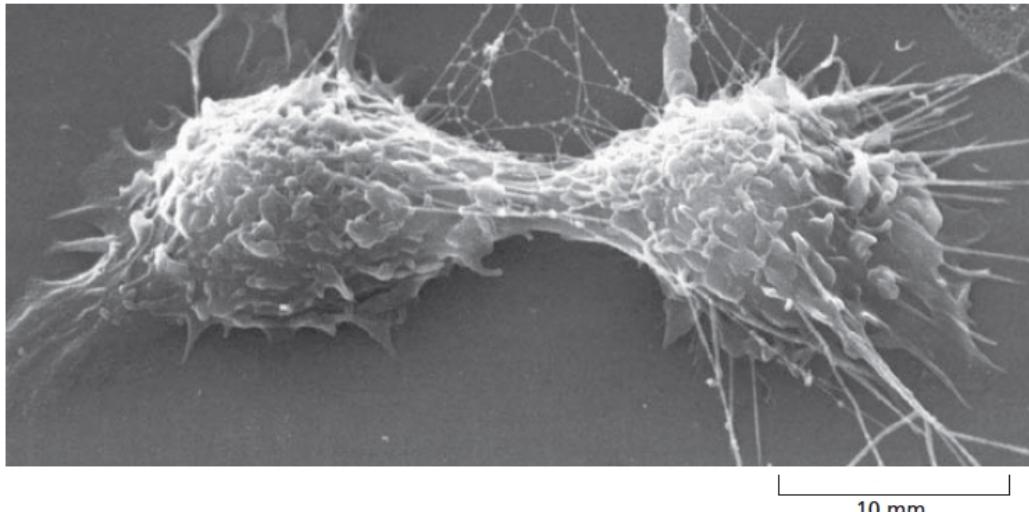


25 μm

Contractile rings underneath the plasma membrane

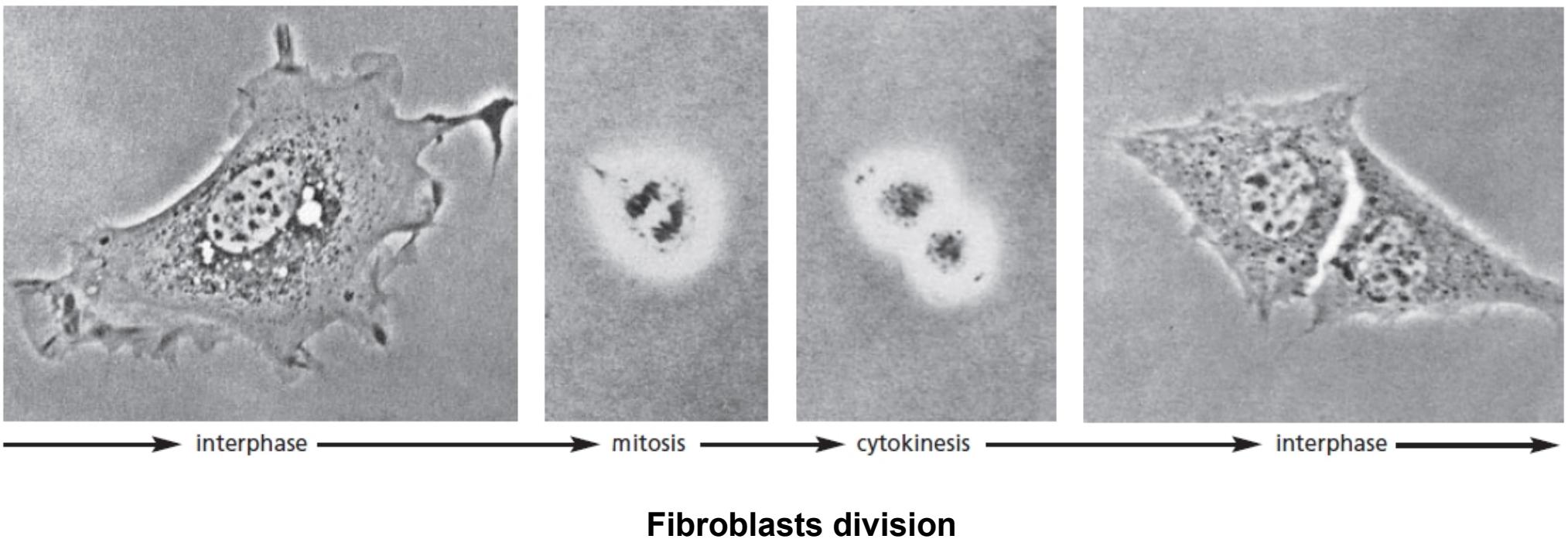
M PHASE STAGES: CYTOKINESIS

- Contractile ring: actin + myosine
- Contractile ring exerts a very strong force



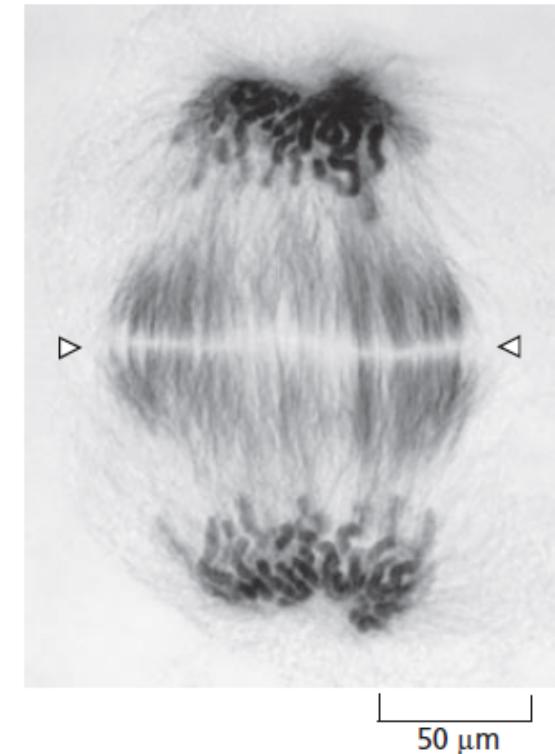
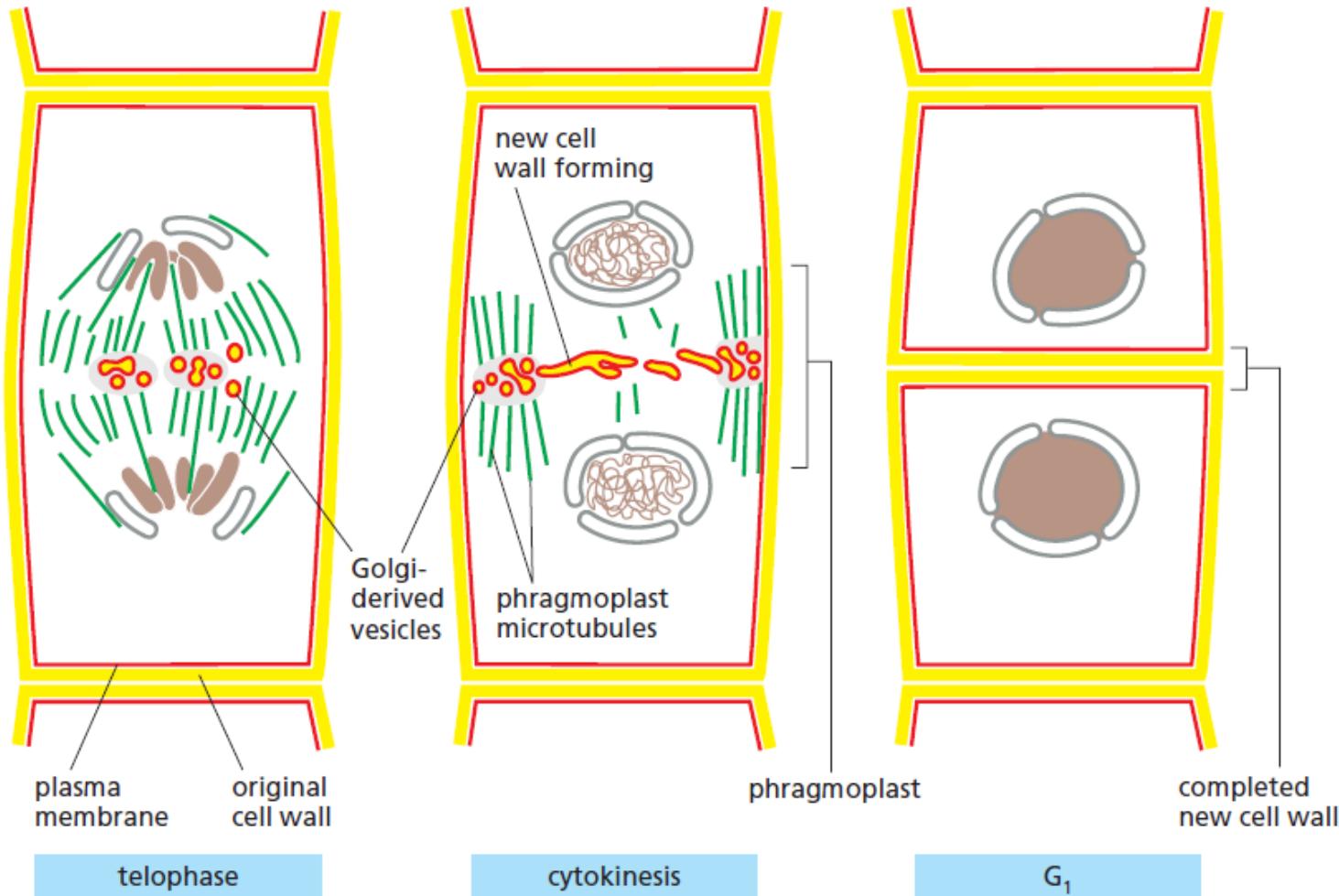
M PHASE STAGES: CYTOKINESIS

Animal cells change the shape in cytokinesis dramatically due to the rebuilding of the cell cortex



CYTOKINESIS AND PLANT CELL WALL

- Remains of interpolar microtubules => phragmoplast
- Phragmoplast accumulate glycoproteins and saccharides from GA using vesicular transport



CYTOKINESIS AND MEMBRANE ENCLOSED MEMBRANES

- Mitochondria, chloroplasts, lysosomes, peroxisomes

- large number

- random distribution

- ER:

- continuous in the interphase

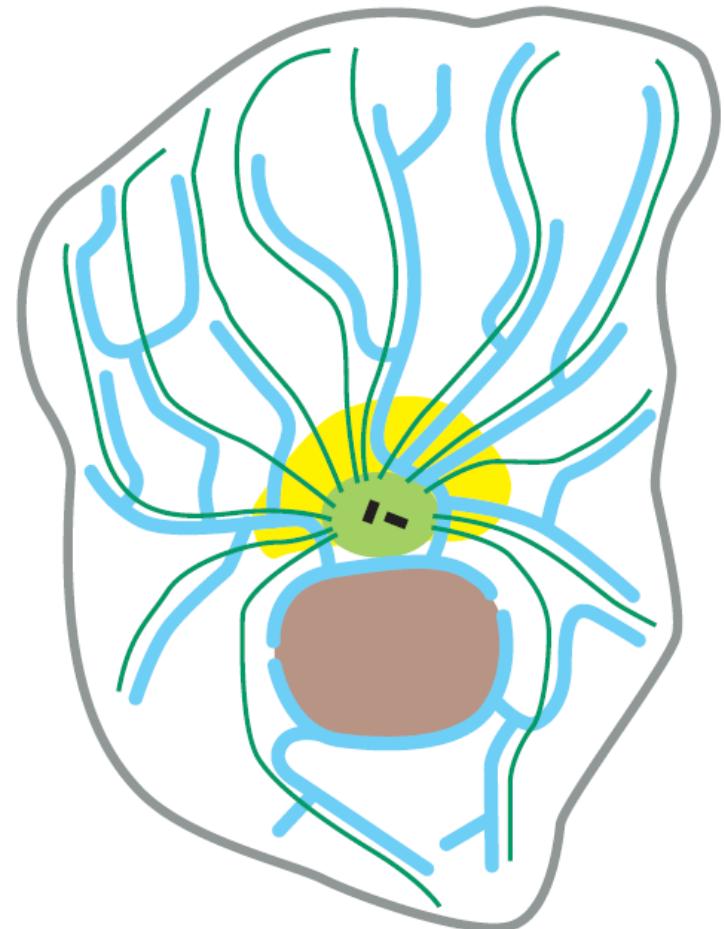
- free and intact in M phase

- cut in two

- GA:

- fragments in the mitosis

- associates with motor proteins



ER

Microtubules

MAJOR CELL CYCLE REGULATORY PROTEINS

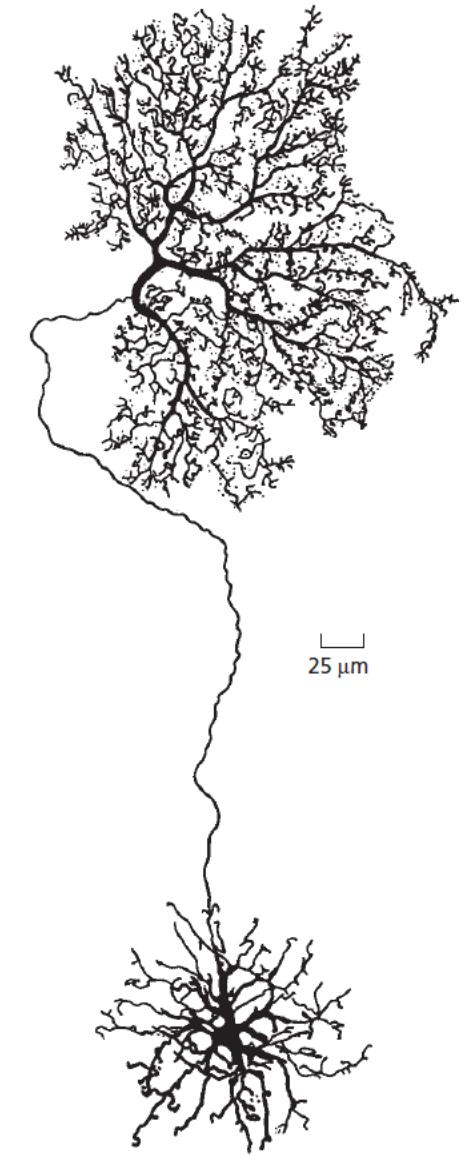
GENERAL NAME	FUNCTIONS AND COMMENTS
Protein kinases and protein	
phosphatases that modify Cdks	
Cdk-activating kinase (CAK)	phosphorylates an activating site in Cdks
Wee1 kinase	phosphorylates inhibitory sites in Cdks; primarily involved in controlling entry into <u>mitosis</u>
Cdc25 phosphatase	removes inhibitory phosphates from Cdks; three family members (Cdc25A, B, C) in mammals; Cdc25C is the activator of Cdk1 at the onset of mitosis
Cdk inhibitory proteins (CKIs)	
Sic1 (budding yeast)	suppresses Cdk activity in G ₁ ; phosphorylation by Cdk1 triggers its destruction
p27 (mammals)	suppresses G ₁ /S-Cdk and S-Cdk activities in G ₁ ; helps cells to withdraw from cell cycle when they terminally differentiate; phosphorylation by Cdk2 triggers its ubiquitylation by SCF
p21 (mammals)	suppresses G ₁ /S-Cdk and S-Cdk activities following DNA damage in G ₁ ; transcriptionally activated by p53
p16 (mammals)	suppresses G ₁ -Cdk activity in G ₁ ; frequently inactivated in cancer
Ubiquitin ligases and their activators	
SCF	catalyzes ubiquitylation of regulatory proteins involved in G ₁ control, including CKIs (Sic1 in budding yeast, p27 in mammals); phosphorylation of target protein usually required for this activity
APC	catalyzes ubiquitylation of regulatory proteins involved primarily in exit from mitosis, including Securin and M-cyclins; regulated by association with activating subunits
Cdc20	APC-activating <u>subunit</u> in all cells; triggers initial activation of APC at <u>metaphase-to- anaphase</u> transition; stimulated by <u>M-Cdk</u> activity
Hct1	maintains APC activity after anaphase and throughout G ₁ ; inhibited by Cdk activity
Gene regulatory proteins	
E2F	promotes transcription of genes required for G ₁ /S progression, including genes encoding G ₁ /S cyclins, S-cyclins, and proteins required for DNA synthesis; stimulated when G ₁ -Cdk phosphorylates Rb in response to extracellular mitogens
p53	promotes transcription of genes that induce cell cycle arrest (especially p21) or <u>apoptosis</u> in response to DNA damage or other cell stress; regulated by association with Mdm2, which promotes p53 degradation

NUMBER AND SIZE OF CELLS

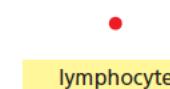
- Cell growth
- Cell division
- Cell death



Organ/organism size



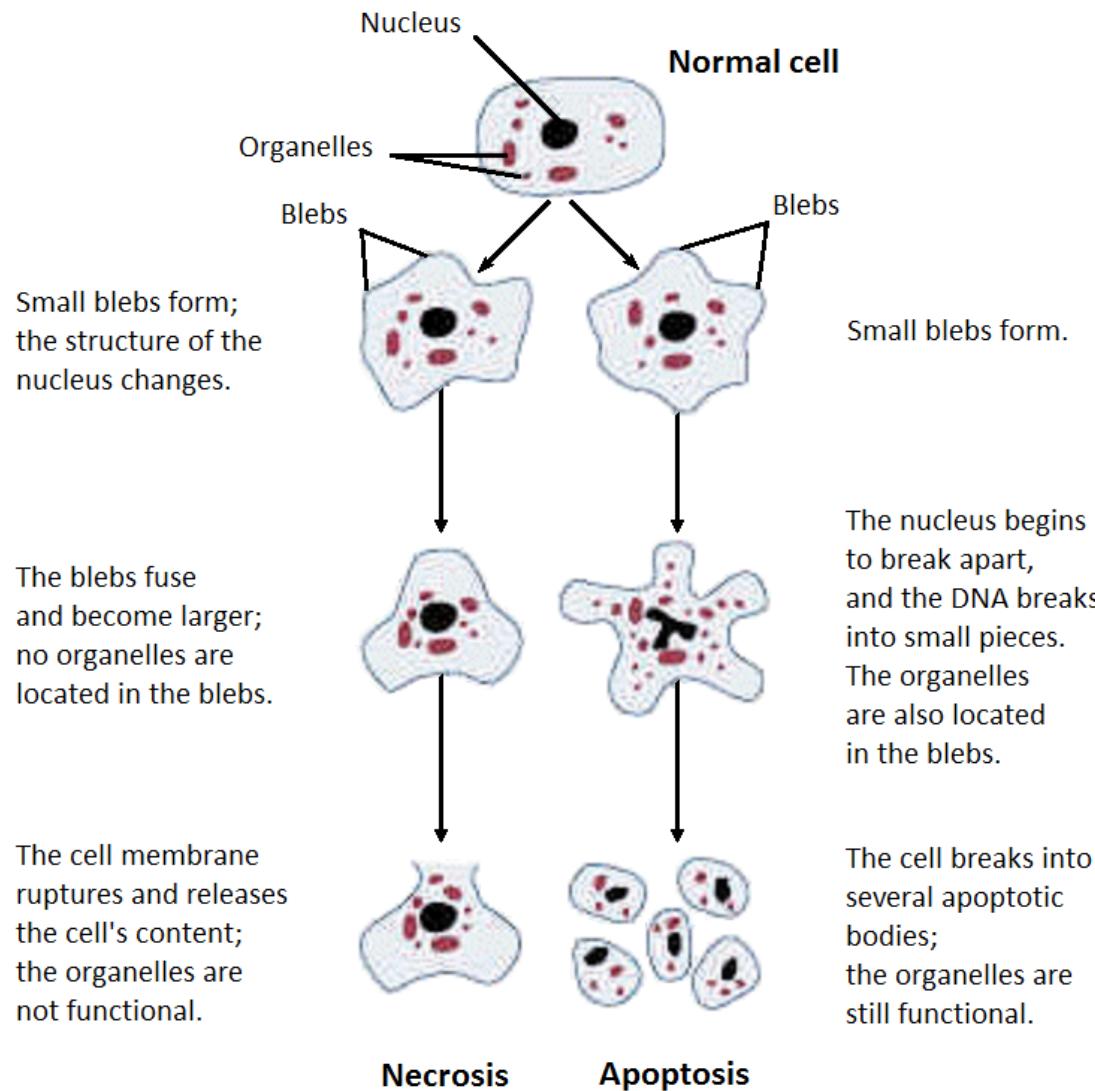
neuron



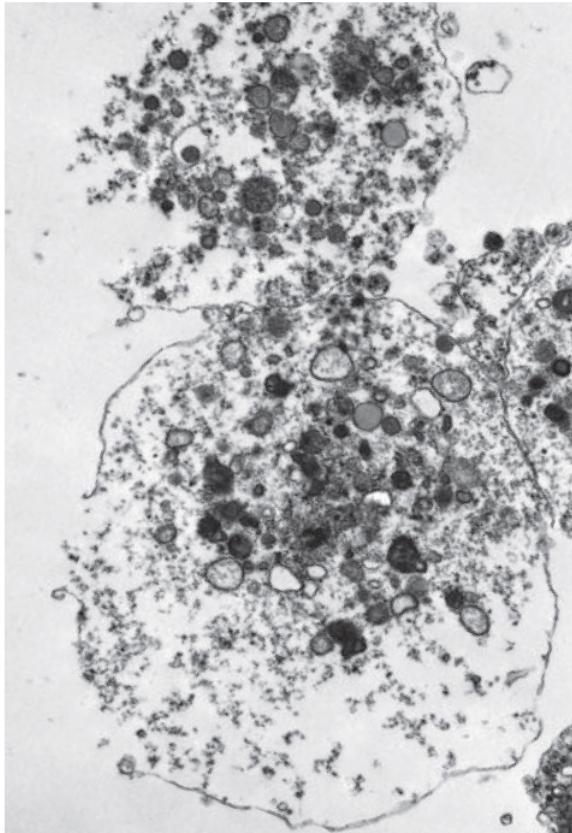
lymphocyte

CELL DEATH: NECROSIS VS. APOPTOSIS

- Not controlled
- Inflammation
- Controlled
- No inflammation



CELL DEATH: NECROSIS VS. APOPTOSIS



Necrosis



Apoptosis



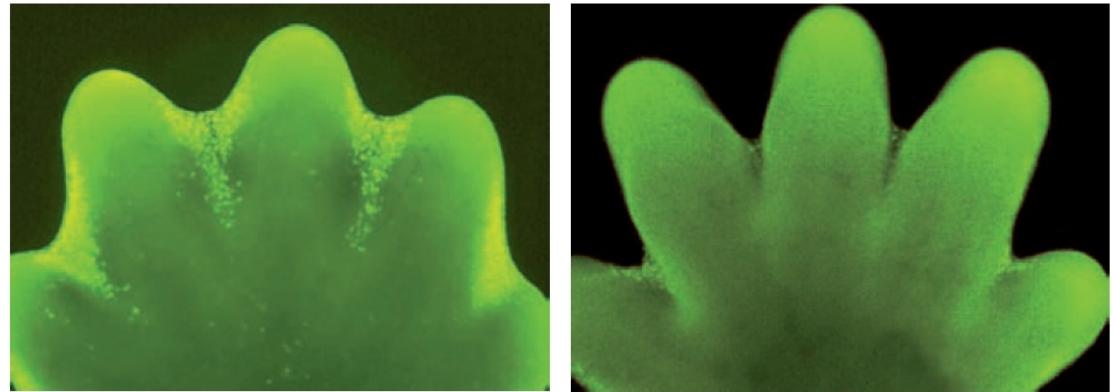
Phagocytosis of an
apoptotic cell

APOPTOSIS

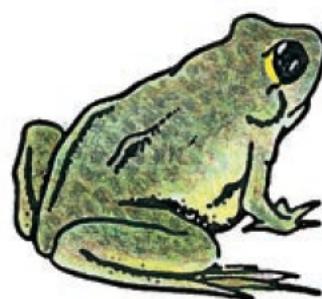
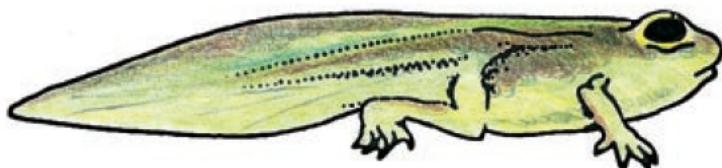
Process of programmed cell death in multicellular organisms

Greek: “falling off”

- Nervous system: half of new cells die very soon after their formation
- 10^9 cells die in human bone marrow/intestine every hour
- Function:
 - unneeded cells are deleted
 - balance with the cell division

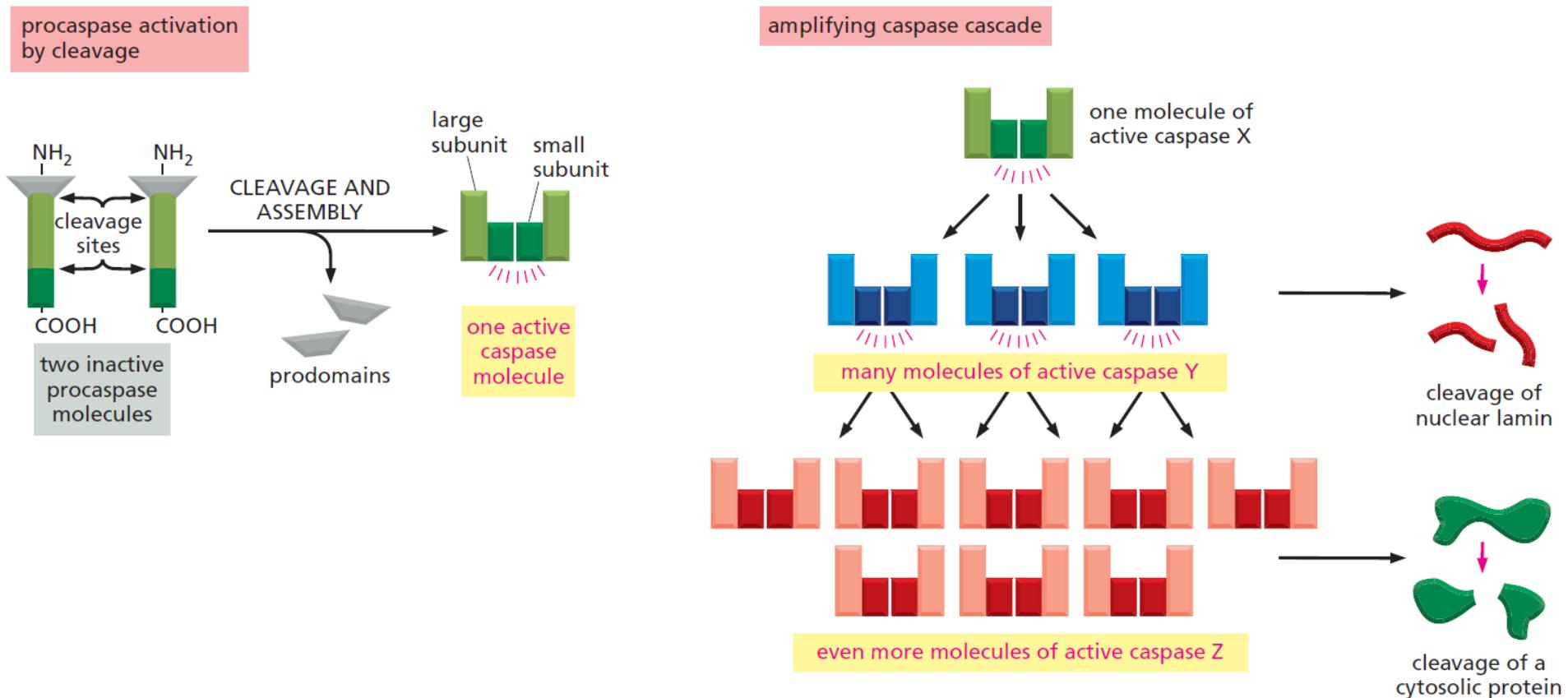


Mouse paw formation
Marker of apoptosis



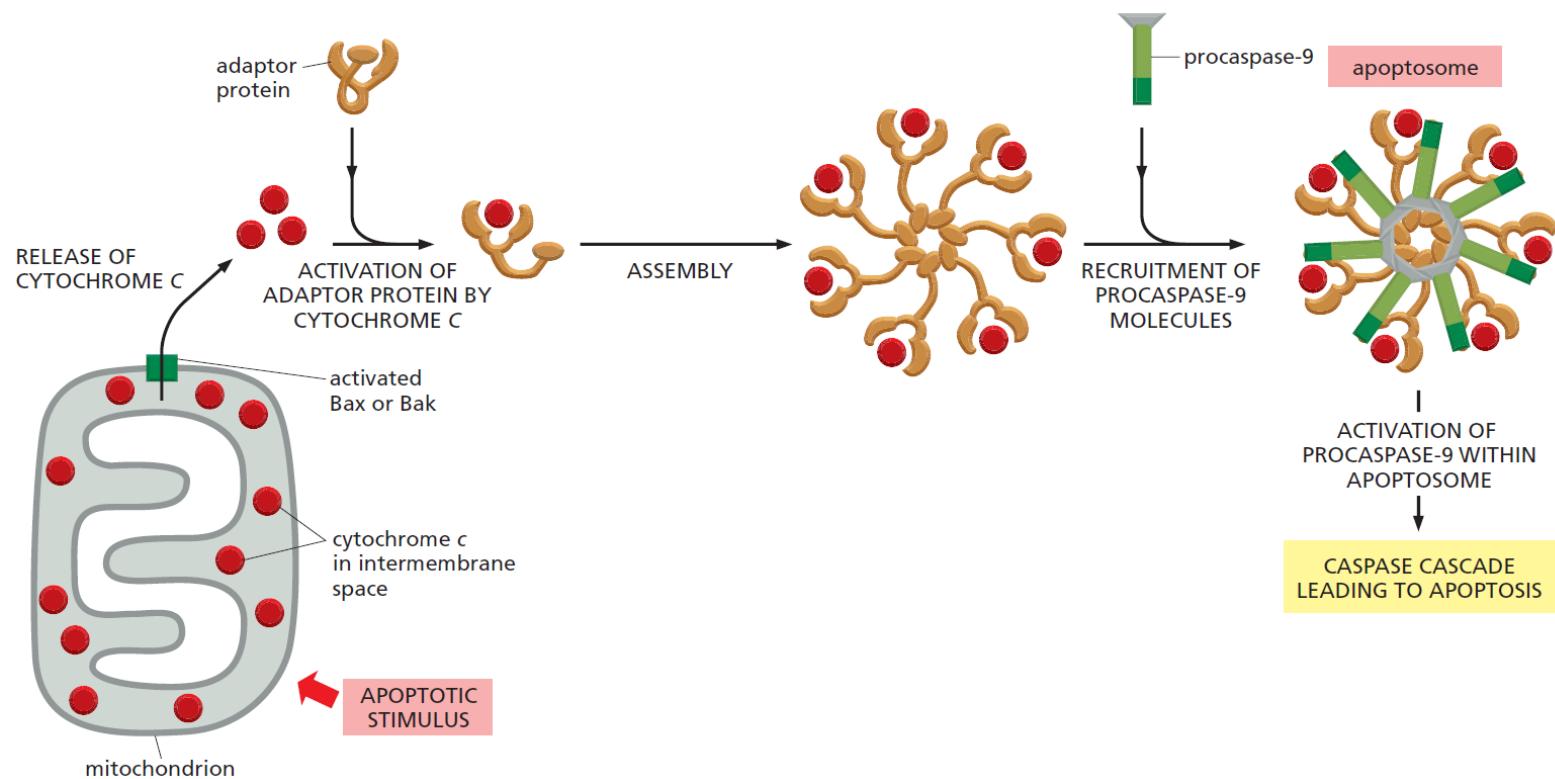
APOPTOSIS: CASPASE PROTEOLYSIS

- Caspase: protease, cascade organization (amplification)
- Procaspsase => activation by apoptotic factors
- Apoptose activation:
 - irreversible
 - “all-or-nothing”
 - strictly controlled



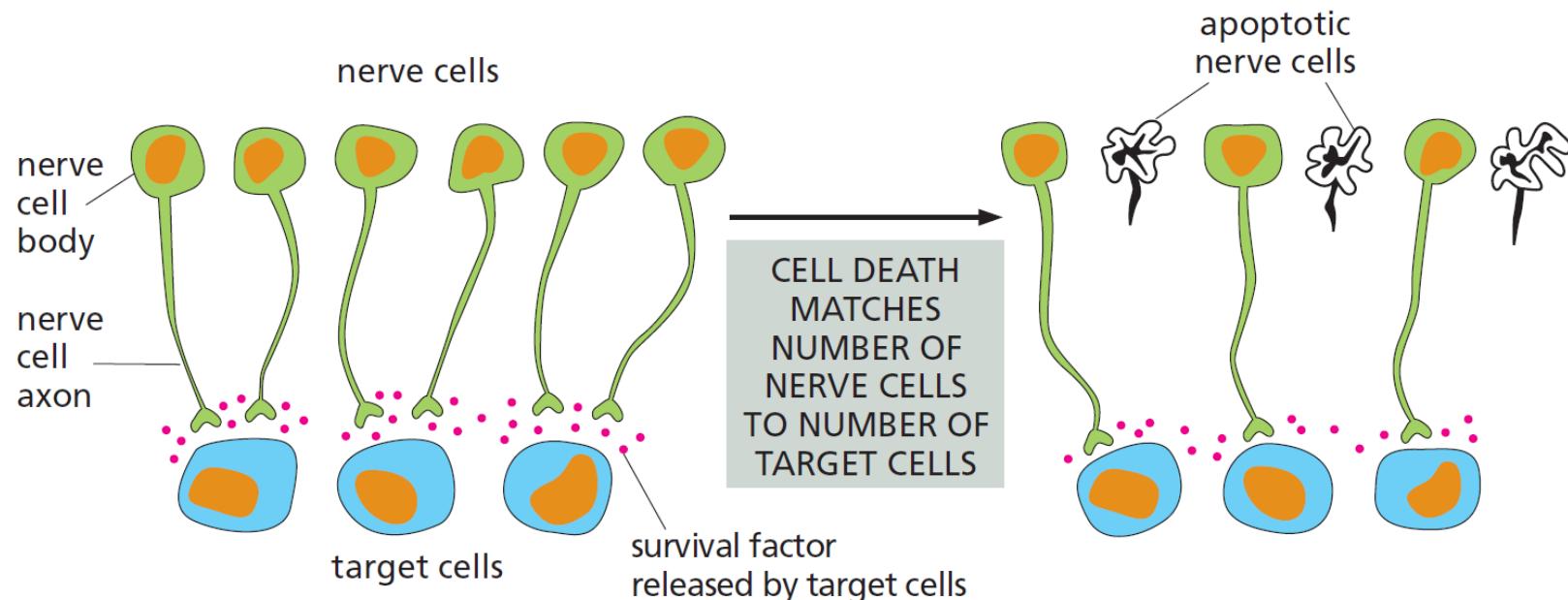
APOPTOSIS: BCL2-FAMILY CONTROL

- Bcl2 family: caspase activation and inhibition
- Bax and Bak: cytochrome C mechanism
- Bax and Bak are regulated by other Bcl2s <= in turn, f.i. DNA damage
- Bcl2: inhibition (apoptosis suppression)

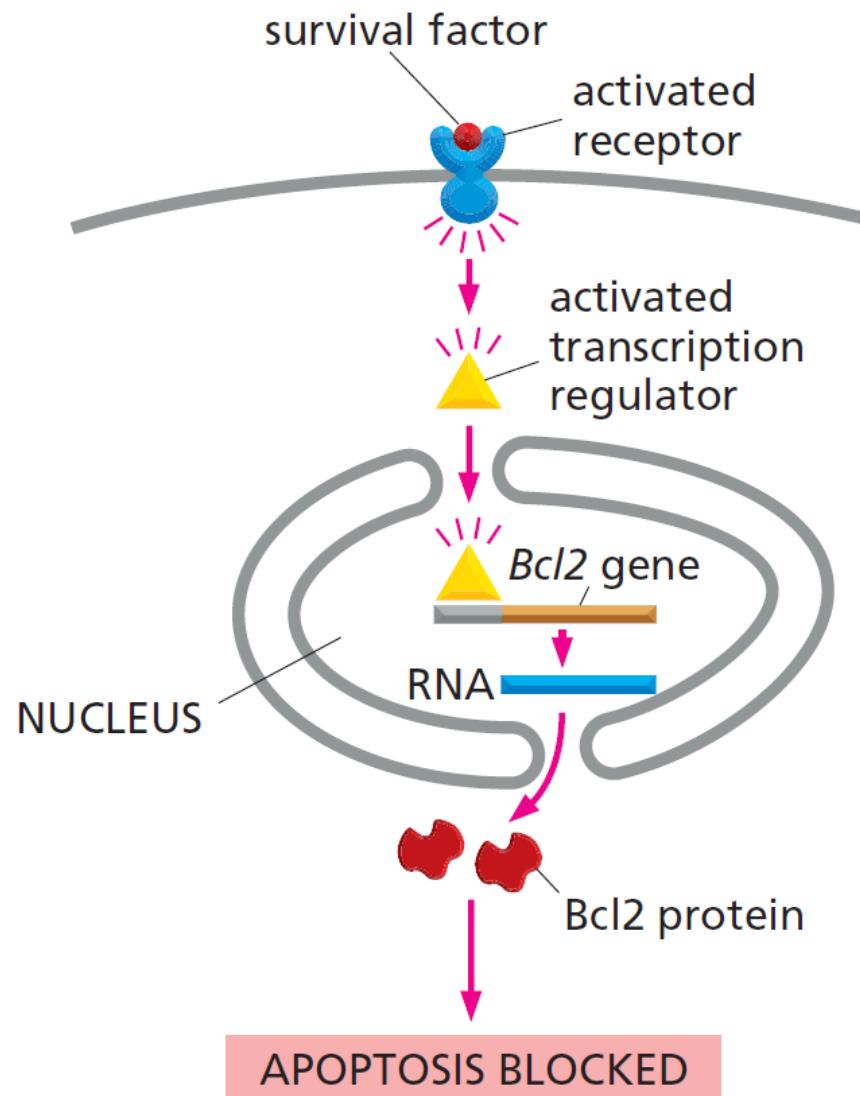


CELL GROWTH AND DIVISION: EXTRACELLULAR FACTORS

- Bacteria and unicellular organisms: nutrients
- Multicellular organisms:
 - nutrients
 - survival factors => cell survival (supression of apoptosis)
 - mitogens => cell division stimulation
 - growth factors => general promotion of synthesis/inhibition of degradation of macromolecules

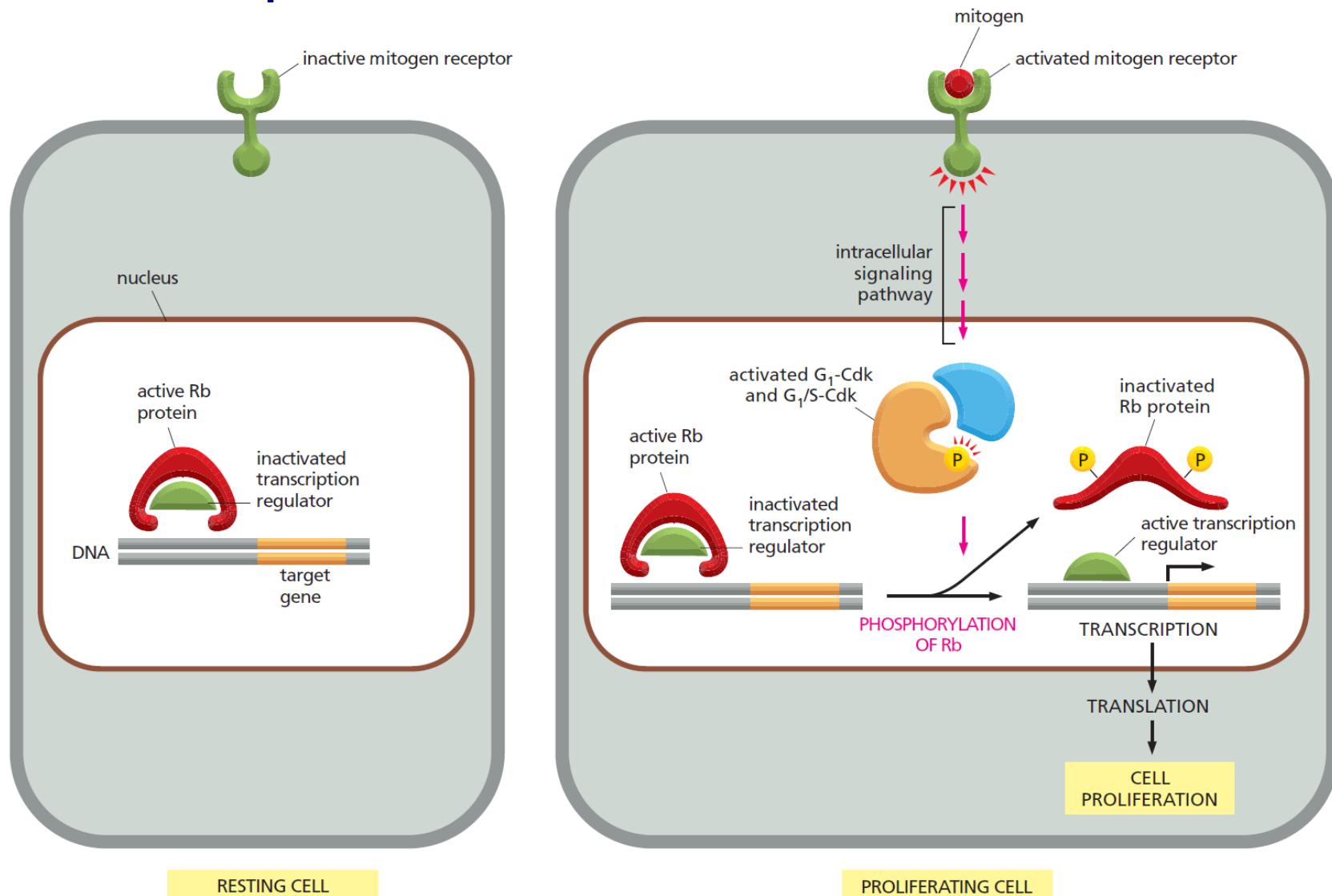


SURVIVAL FACTORS ARE NEEDED TO AVOID APOPTOSIS



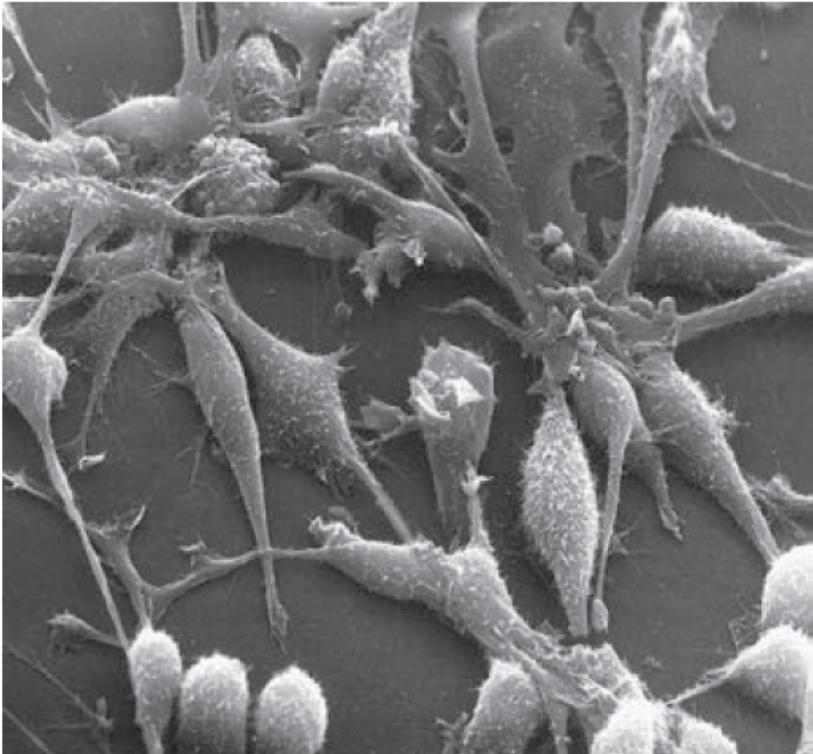
MITOGENS STIMULATE CELL DIVISION

- Binding to cell receptors
- Activating the pathways releasing brakes for G1=>S transition
- Retinoblastoma protein



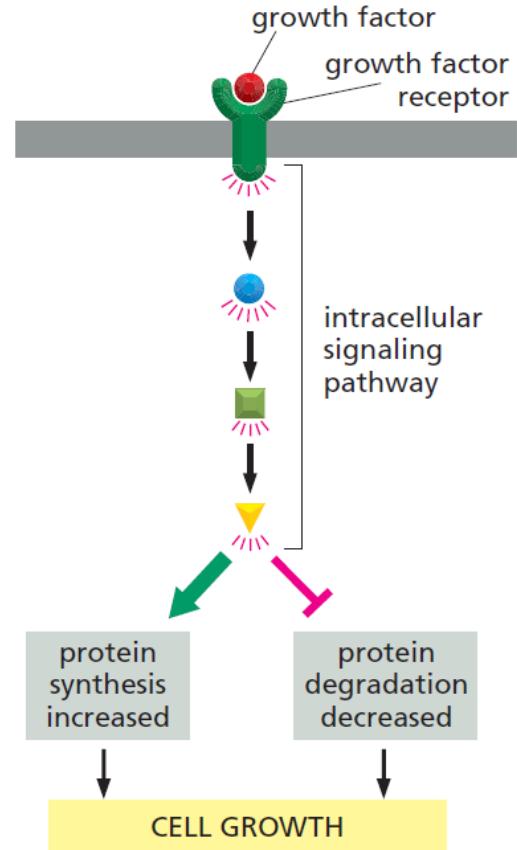
GROWTH FACTORS INDUCE CELL GROWTH

- Highly cell type specific (f.i.: platelet-derived GF, hepatocyte GF)
- Mechanism:
 - synthesis activation
 - degradation inhibition
- Some are also mitogens



Rat fibroblasts proliferation

10 μm



INHIBITION OF CELL GROWTH AND PROLIFERATION

- Myostatin: growth differentiation factor 8



LECTURES 21-22: CELL CYCLE I

- Introduction to the cell cycle: principles and discoveries
- Phases of cell cycle and their control:
 - interphase (S , G1 , G2 phases)
 - M phase (mitosis, cytokinesis)
- Apoptosis

