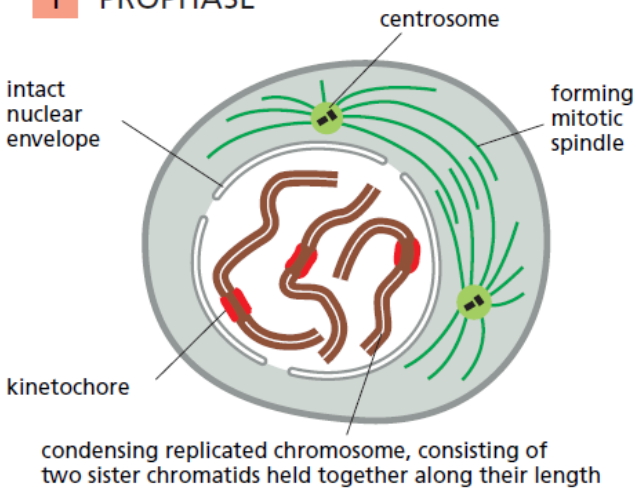
Lecture 15. Cell cycle II

Outline

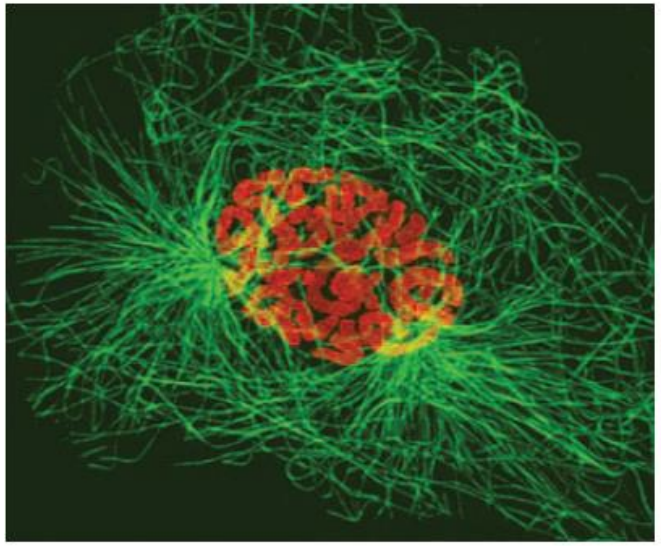
I. Mitosis
II. Cytokinesis
III. Control of cell division and cell growth

IV. Mitosis

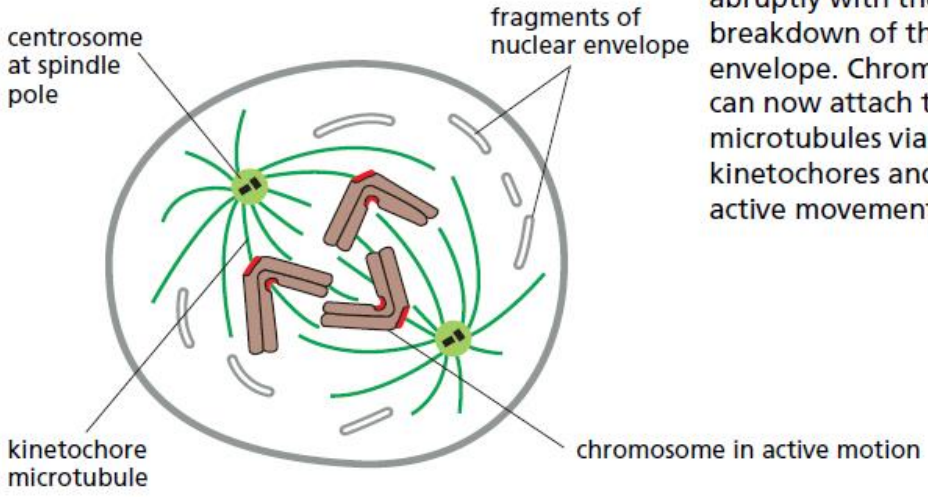
1 PROPHASE



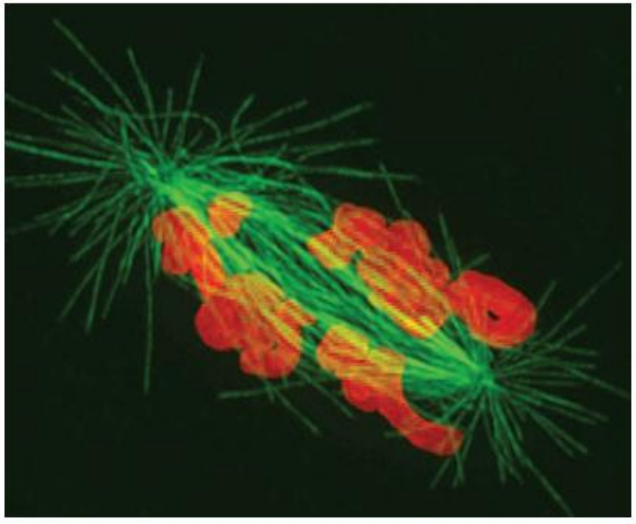
At prophase, the replicated chromosomes, each consisting of two closely associated sister chromatids, condense. Outside the nucleus, the mitotic spindle assembles between the two centrosomes, which have replicated and moved apart. For simplicity, only three chromosomes are shown. In diploid cells, there would be two copies of each chromosome present. In the photomicrograph, chromosomes are stained *orange* and microtubules are *green.*



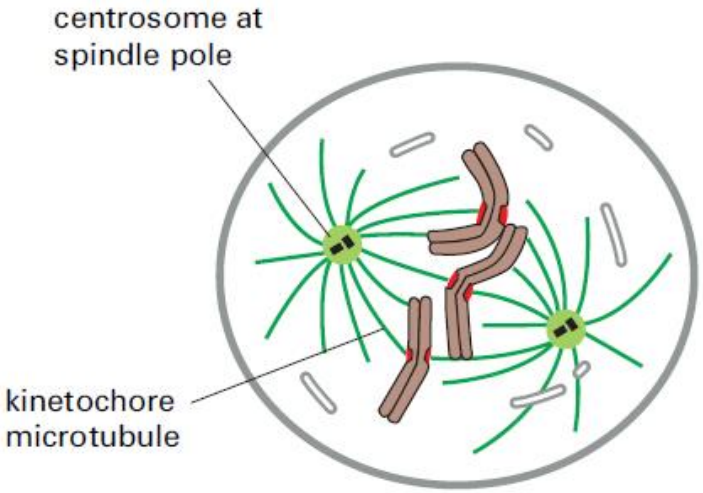
2 PROMETAPHASE



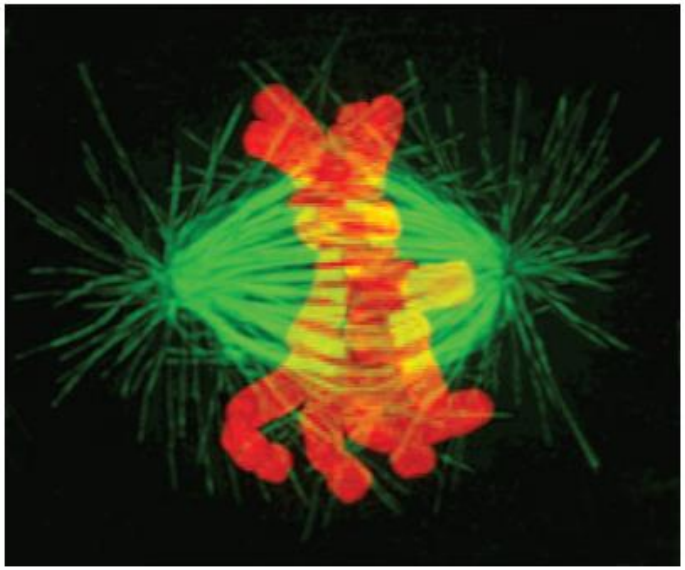
Prometaphase starts abruptly with the breakdown of the nuclear envelope. Chromosomes can now attach to spindle microtubules via their kinetochores and undergo active movement.



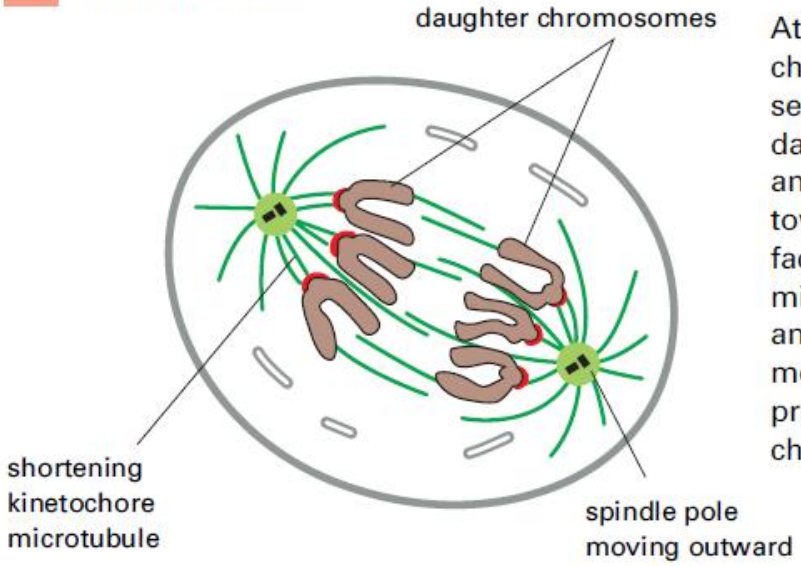
3 METAPHASE



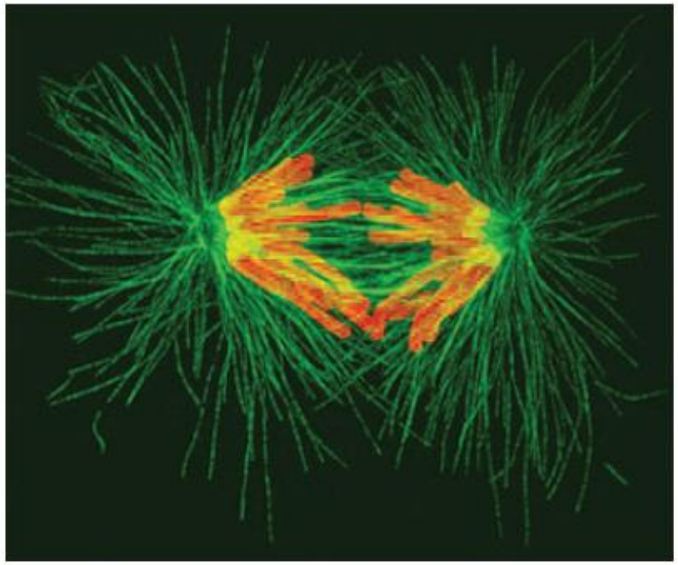
At metaphase, the chromosomes are aligned at the equator of the spindle, midway between the spindle poles. The kinetochore microtubules attach sister chromatids to opposite poles of the spindle.



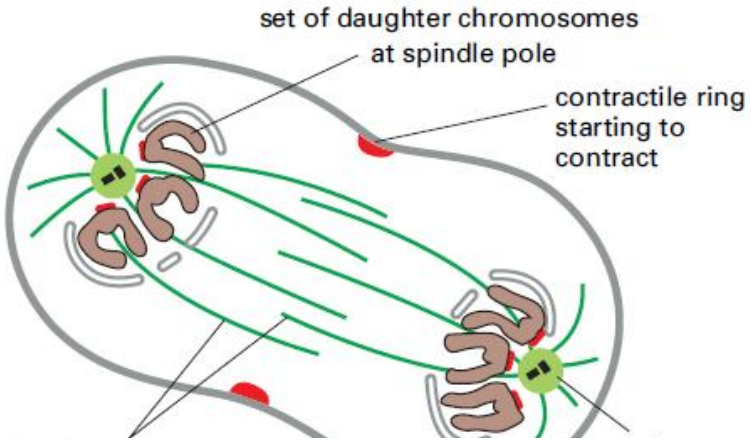
4 ANAPHASE



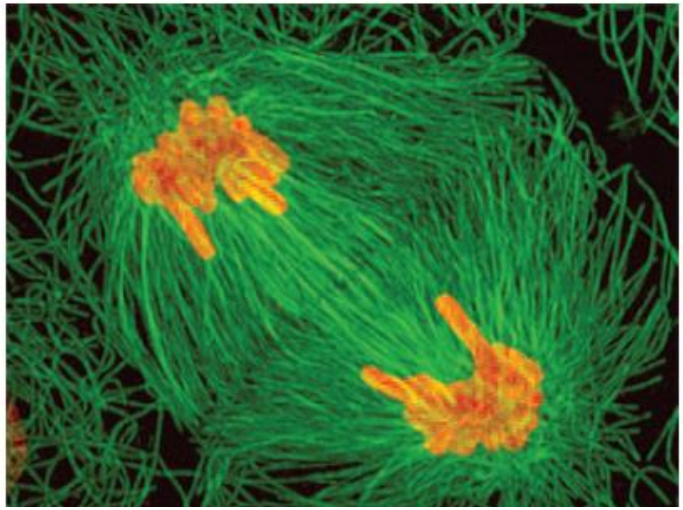
At anaphase, the sister chromatids synchronously separate to form two daughter chromosomes, and each is pulled slowly toward the spindle pole it faces. The kinetochore microtubules get shorter, and the spindle poles also move apart; both processes contribute to chromosome segregation.



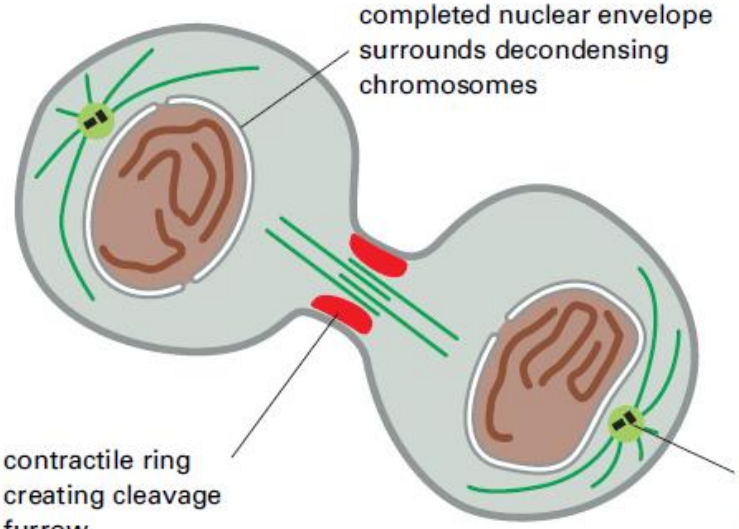
5 TELOPHASE



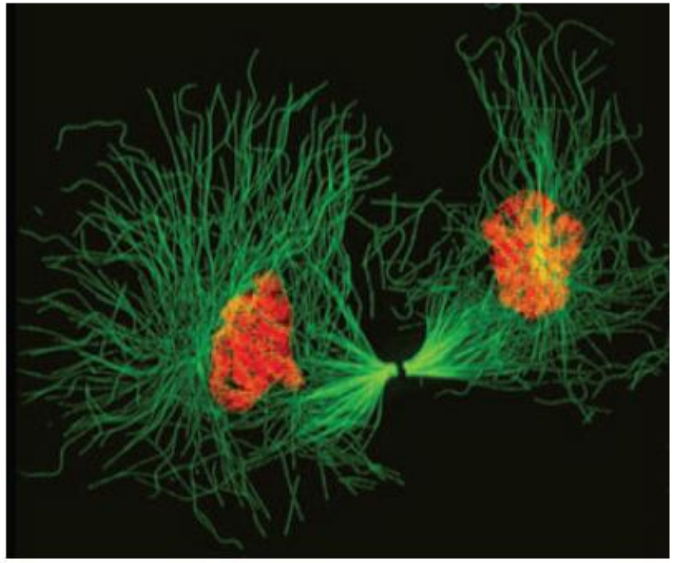
During telophase, the two sets of daughter chromosomes arrive at the poles of the spindle and decondense. A new nuclear envelope reassembles around each set, completing the formation of two nuclei and marking the end of mitosis. The division of the cytoplasm begins with contraction of the contractile ring.



6 CYTOKINESIS



During cytokinesis, the cytoplasm is divided in two by a contractile ring of actin and myosin filaments, which pinches the cell in two to create two daughters, each with one nucleus.



(Micrographs courtesy of Julie Canman and Ted Salmon.)

Mitosis can be divided into two phases

- 1. From Prophase, prometaphase to metaphase, M-Cdk and several other mitotic protein kinases phosphorylate a variety of proteins leading to assembly of the mitotic spindle and its attachment to the sister chromatids pairs
- 2. Metaphase to Anaphase transition, APC/C triggers the cleavage of cohesions and initiates segregation of sister chromatids . APC/C also triggers the destruction of cyclins, deactivate cdks, disassembly of mitotic spindle.

17.4-animal_cell_division.mov

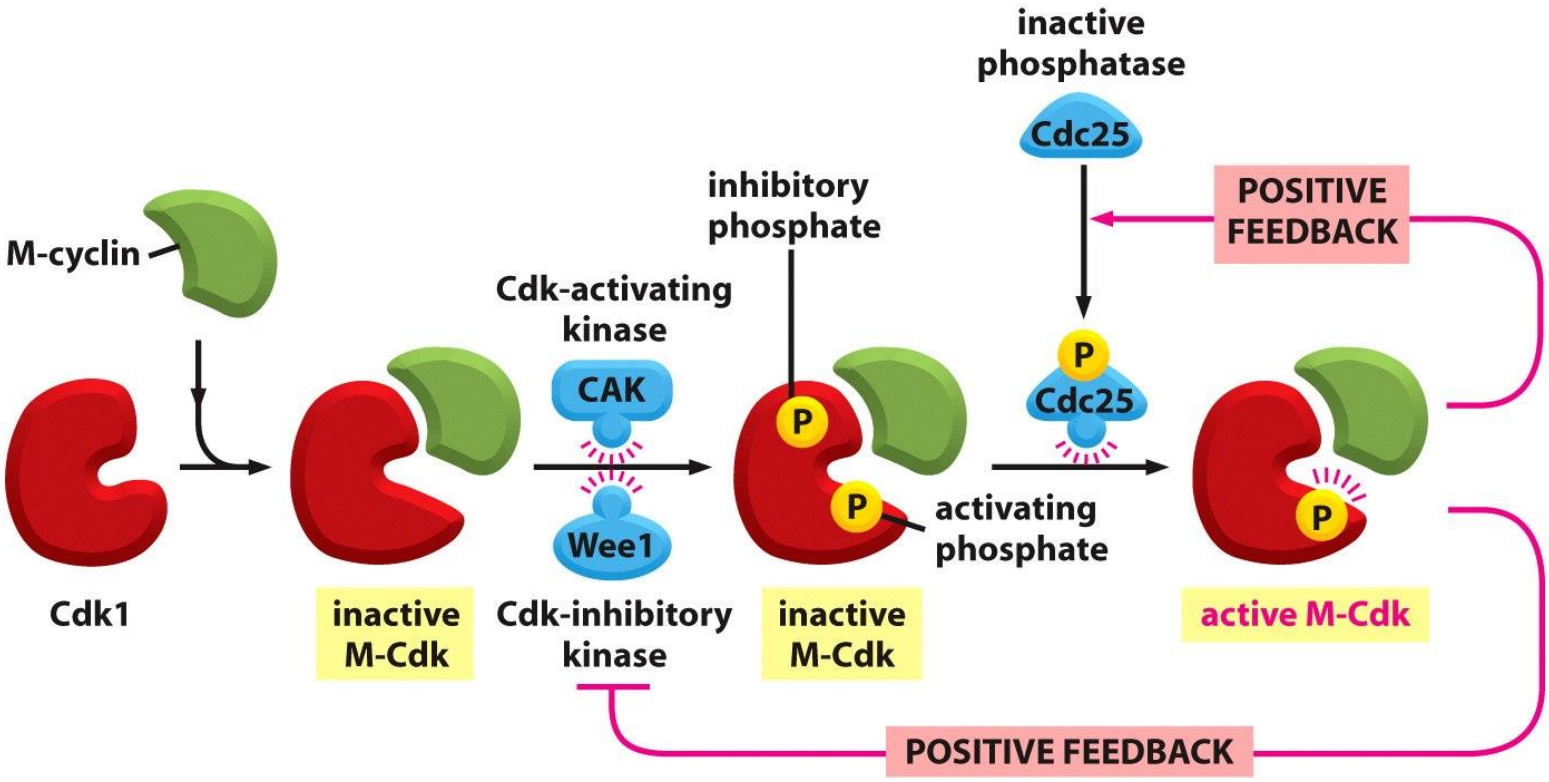
Early in Mitosis

M-Cdk and its effectors:

- 1. Induce assembly of mitotic spindle and its attachment to the sister chromatids pairs.
- 2. Trigger chromosome condensation.
- 3. Breakdown of nuclear envelope
- 4. Rearrangement of the actin cytoskeleton and Golgi apparatus.

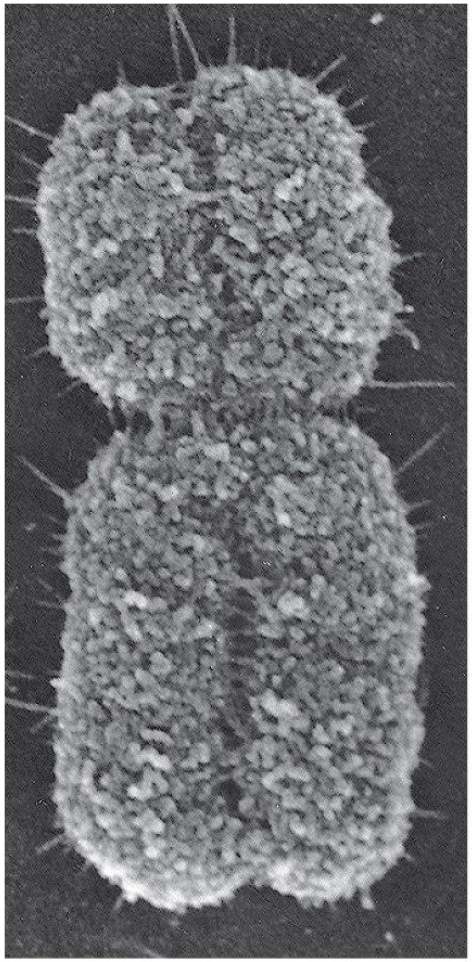
How? Not fully understood, not all substrates for M-Cdk have been identified

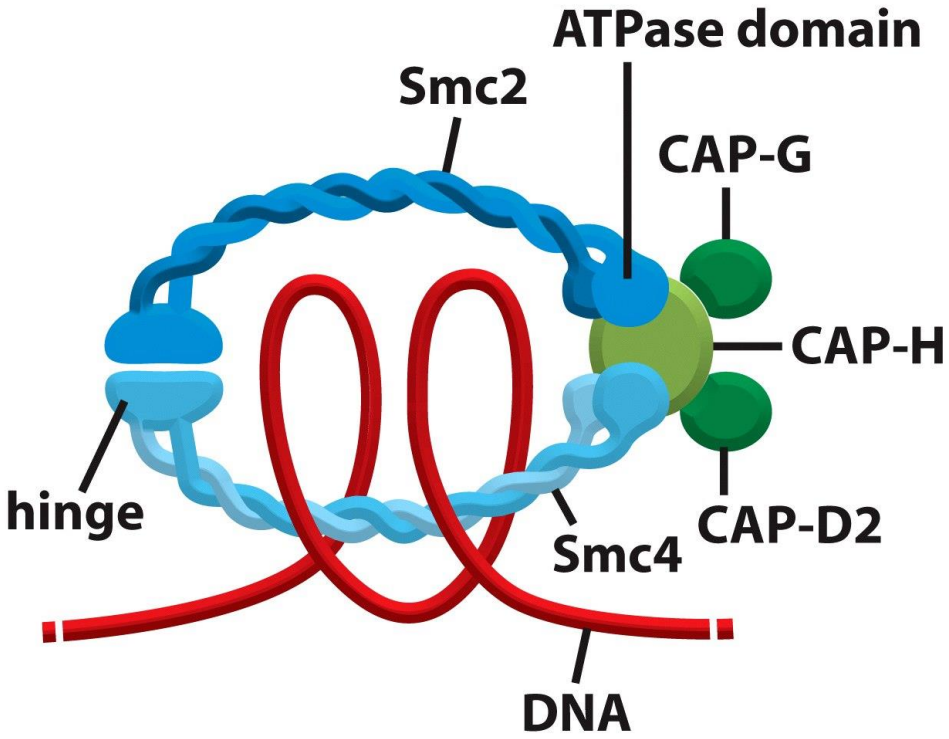
How is M-Cdk activated?



At the onset of mitosis, it is activated by cdc25.

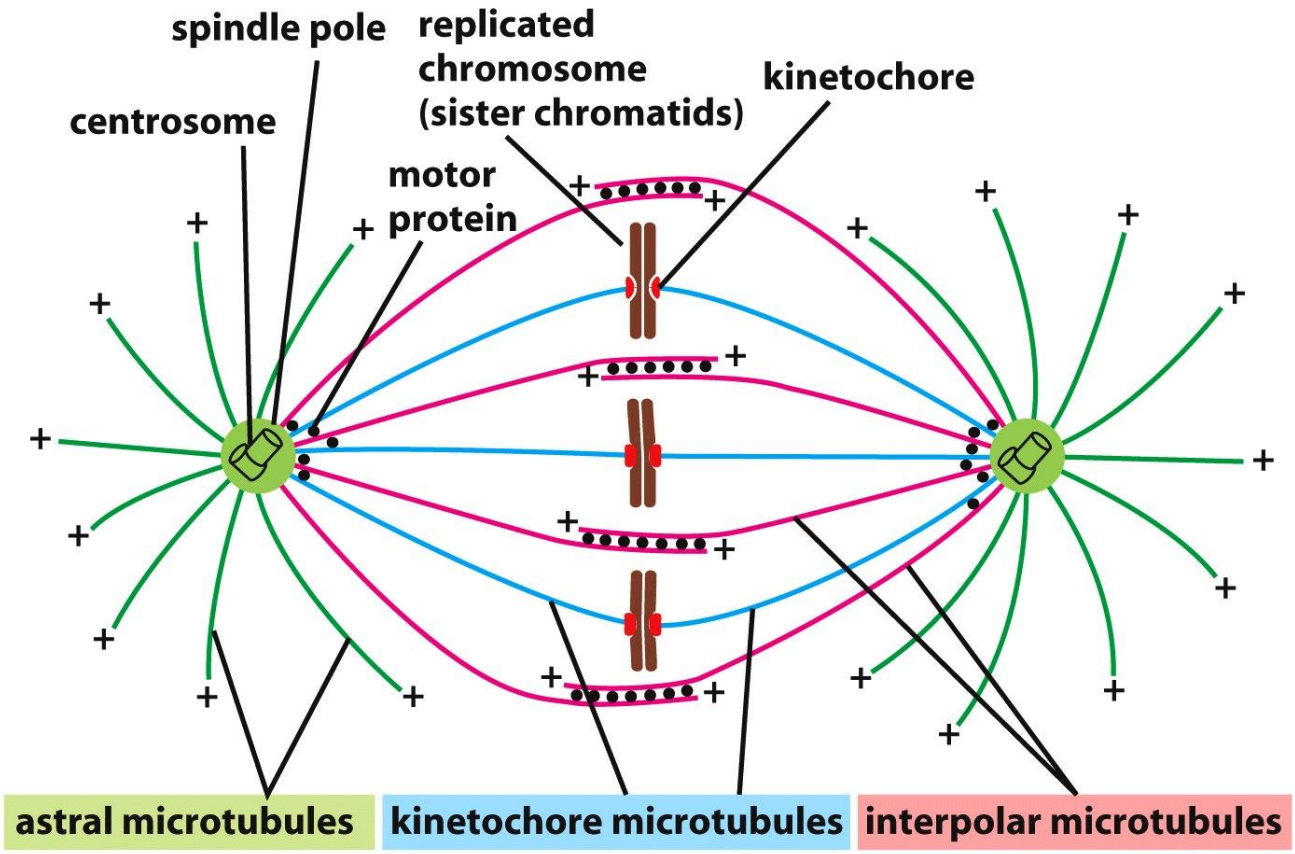
Sister chromatin condensation by condensins---phosphorylated and activated by M-Cdk





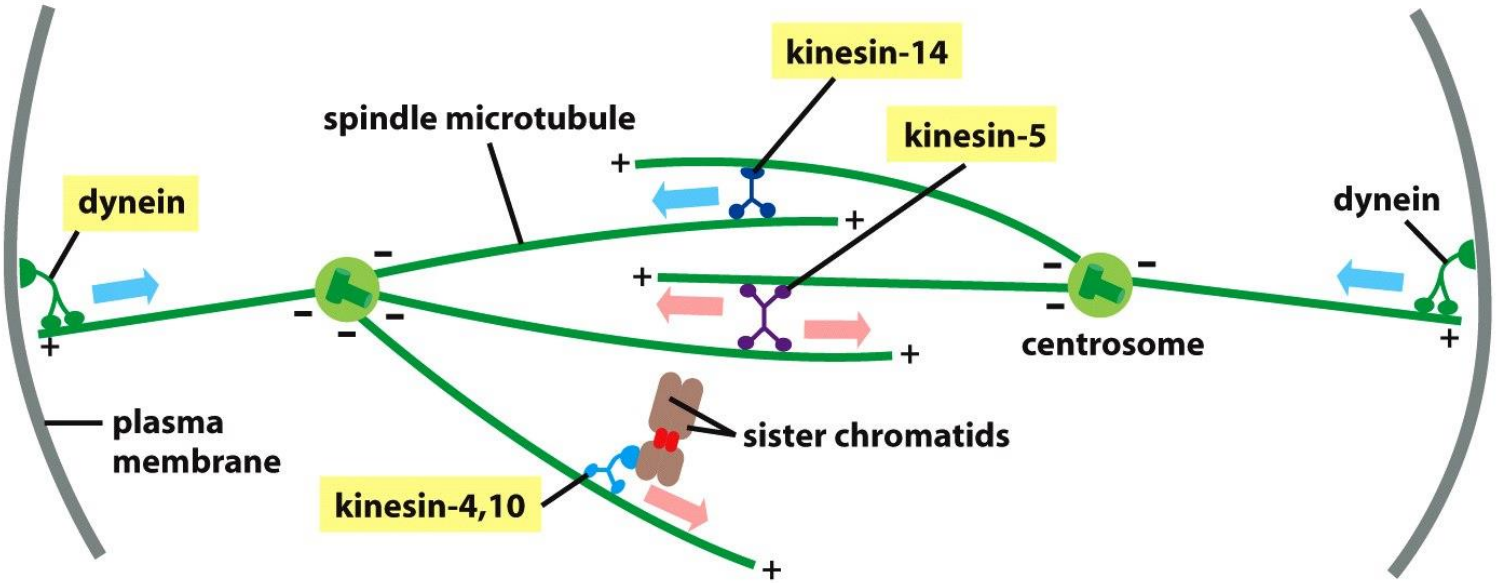
M-Cdk also triggers formation of mitotic spindle

three classes of microtubules:



Microtubule-dependent motor proteins govern spindle assembly and function

- Kinesin-5: push poles apart
- Kinesin-14: pull poles together
- Kinesins-4,-10: chromokinesins, push the attached chromosomes away from the pole
- Dynein: pull spindle poles away from each other



Formation of spindle

- Two mechanisms ensure bipolarity of spindles

1)Centrosome-dependent

2)Self-organization, Chromosome and motor proteins-dependent

17.7-mitotic_spindle.mov

1). Centrosome-dependent spindle assembly

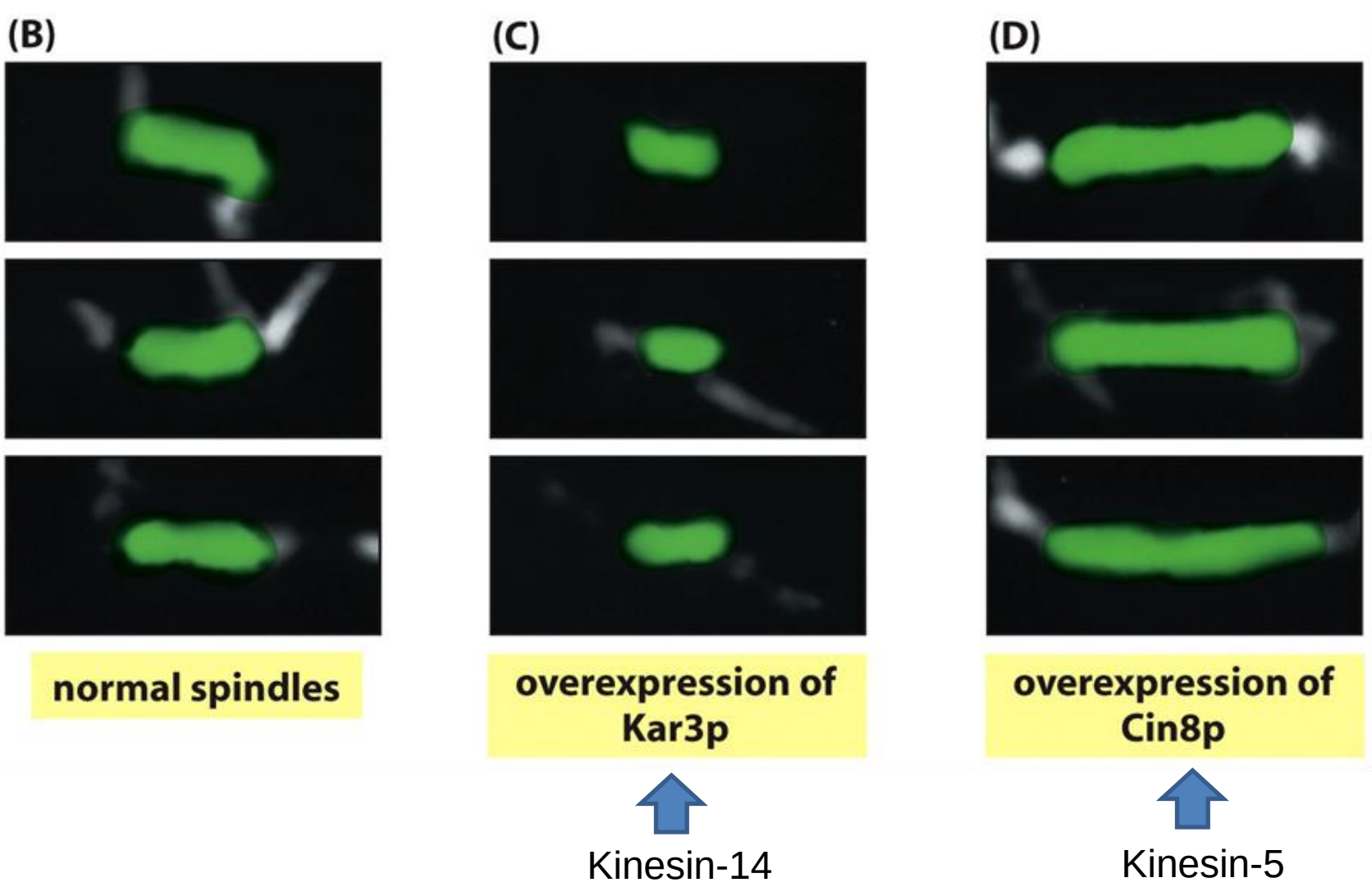
- M-cdk promotes centrosome maturation---amount of γ-tubulin ring complex increases, more activities in nucleating microtubules.
- M-cdk and other mitotic protein kinases promotes centrosome separation:

  dynein: pull centrosomes away from each other

  kinesin-5: push centrosomes away from each other

  kinesin-14 : pull centrosomes together
- A balance reaches for all the activities to determine the final length of spindles.

Experiments to show the opposing effects from kinesin-14 and kinesin-5 on spindle length



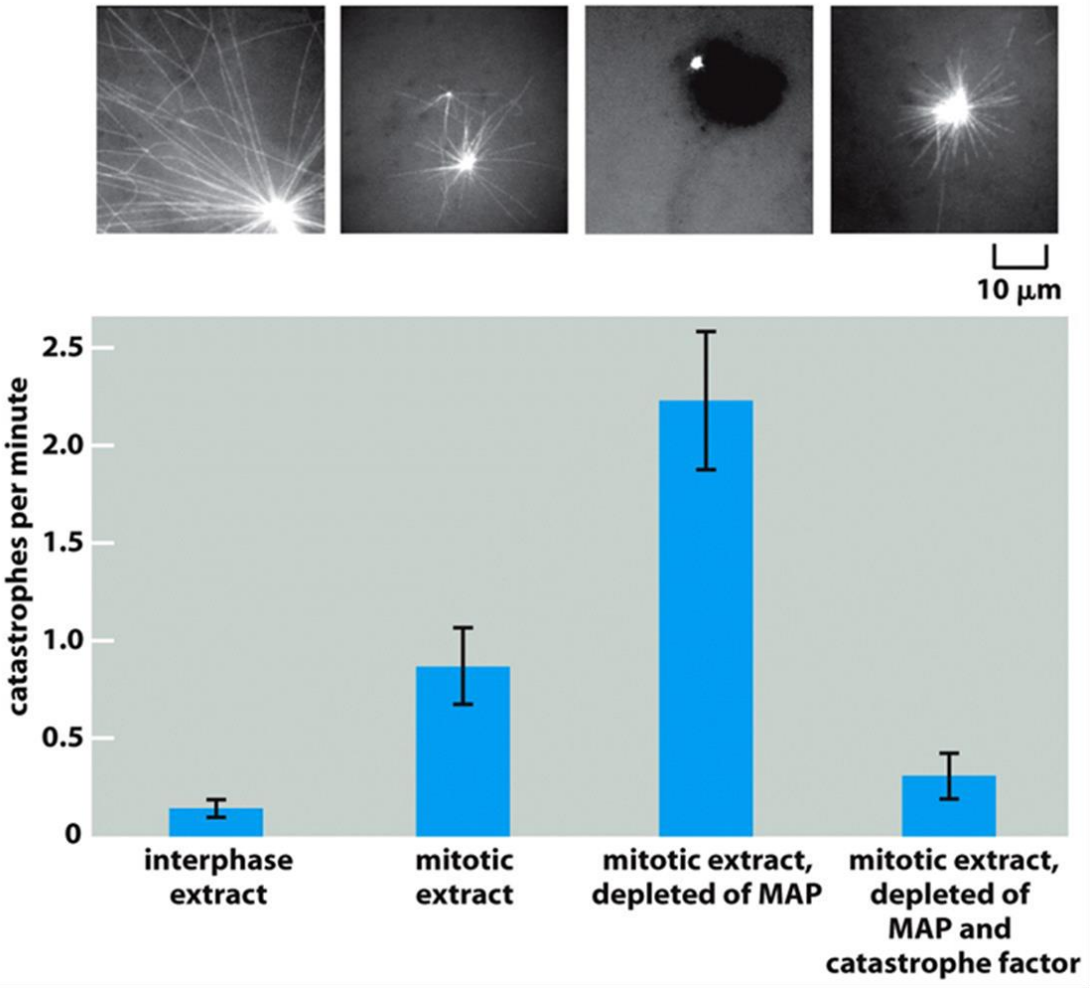
Nuclear envelope disassembles

- M-cdk and mitotic protein kinases phosphorylate several components in nuclear pore complex and lamin, promotes the disassembly of the nuclear envelope and nuclear lamina.

Microtubule instability increases greatly in mitosis

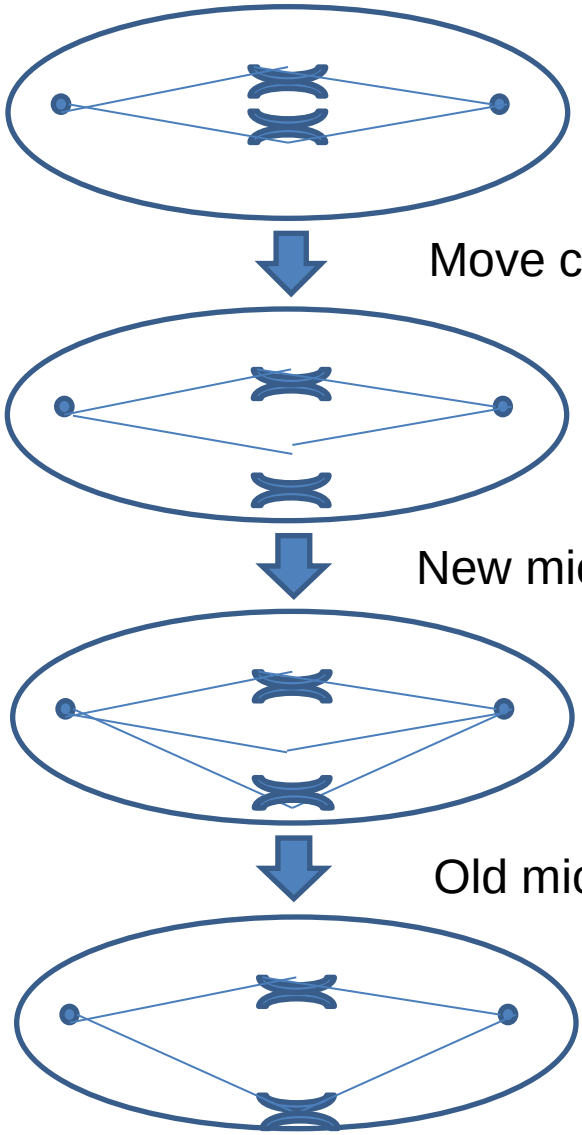
- Two mechanisms: catastrophe factors destabilizes microtubule arrays
- Microtubule-associated proteins (MAPs): stabilized microtubule arrays

Experimental evidence to show the balance between catastrophe factors and MAPs on microtubule length



2). Mitotic chromosomes promotes bipolar spindle assembly

Evidence from Experiments:



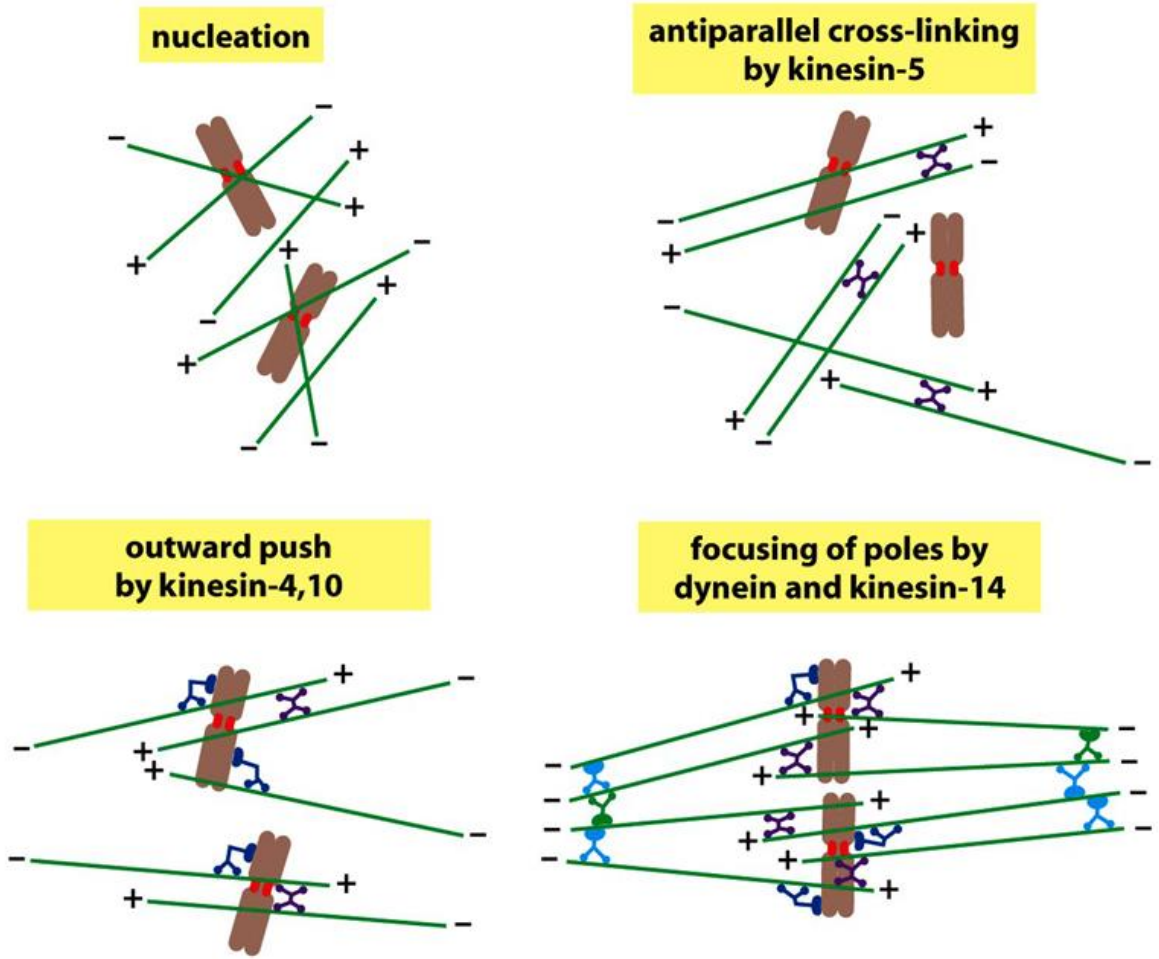
Detailed mechanism for acentrosomal spindle assembly

- 1. Chromosome bound GEF activates Ran-GTP, which then releases microtubule-stabilizing proteins and stimulate local nucleation and stabilization of microtubules around chromosomes.
- 2. kinesin-5 crosslinks microtubules and push minus ends toward the spindle poles.

kinesins -4 and -10 push the minus ends away from the chromosomes

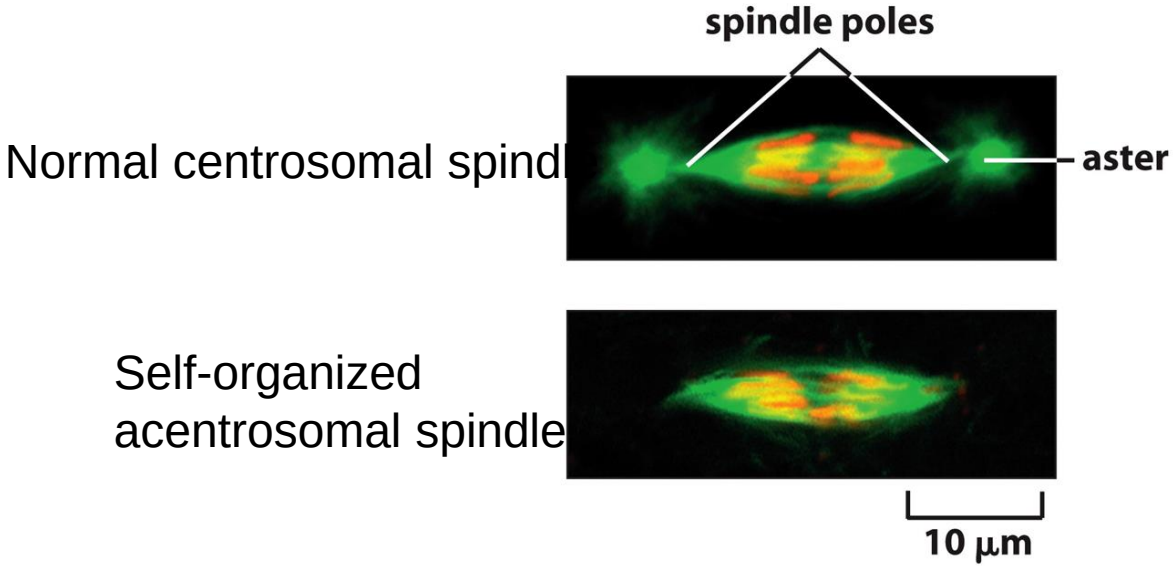
Dynein, kinsin-14, cross-link and focus and minus ends to form two spindle poles.

Spindle self-organization by motor proteins



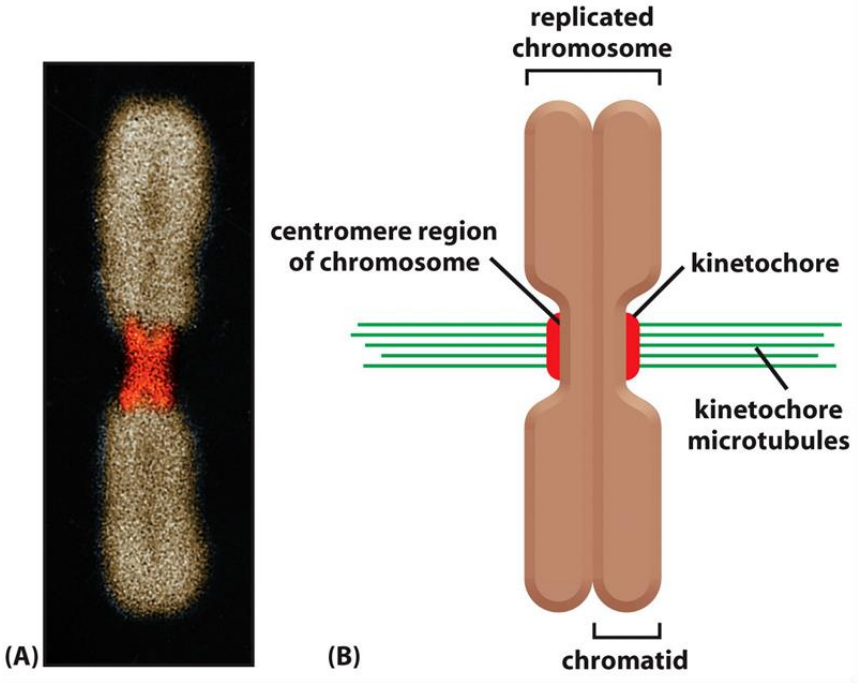
Self-organization of spindle

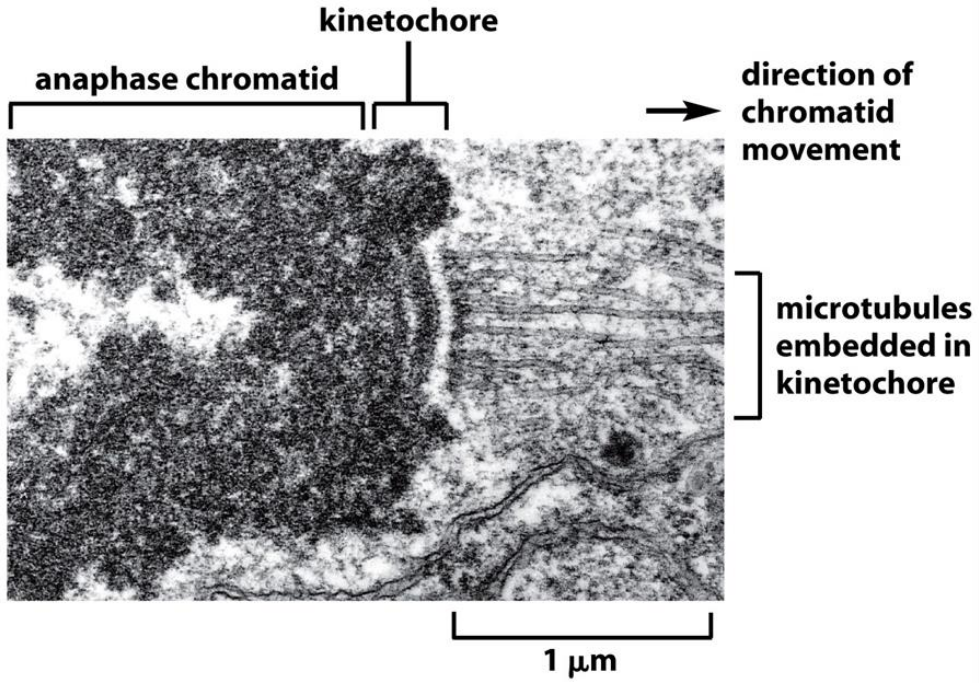
- Exists in Higher plants
- Exists in Many animal oocytes
- Certain insect embryos without fertilization
- However in animal cells, acentrosomal spindle is often mis-positioned and results in abnormalities in cytokinesis



Kinetochores attach sister chromatids to the spindle

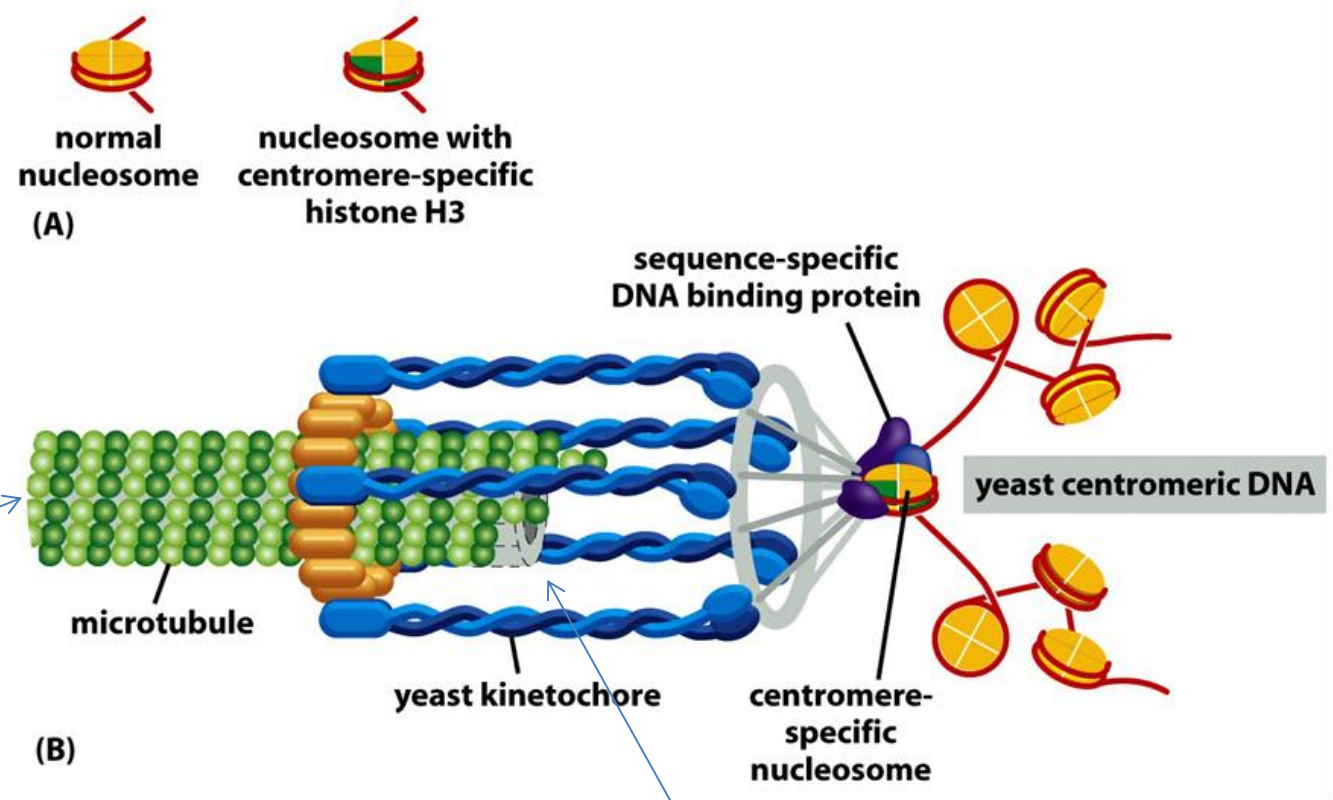
- Structure of kinetochores





Kinetochore is the microtubule attachment site

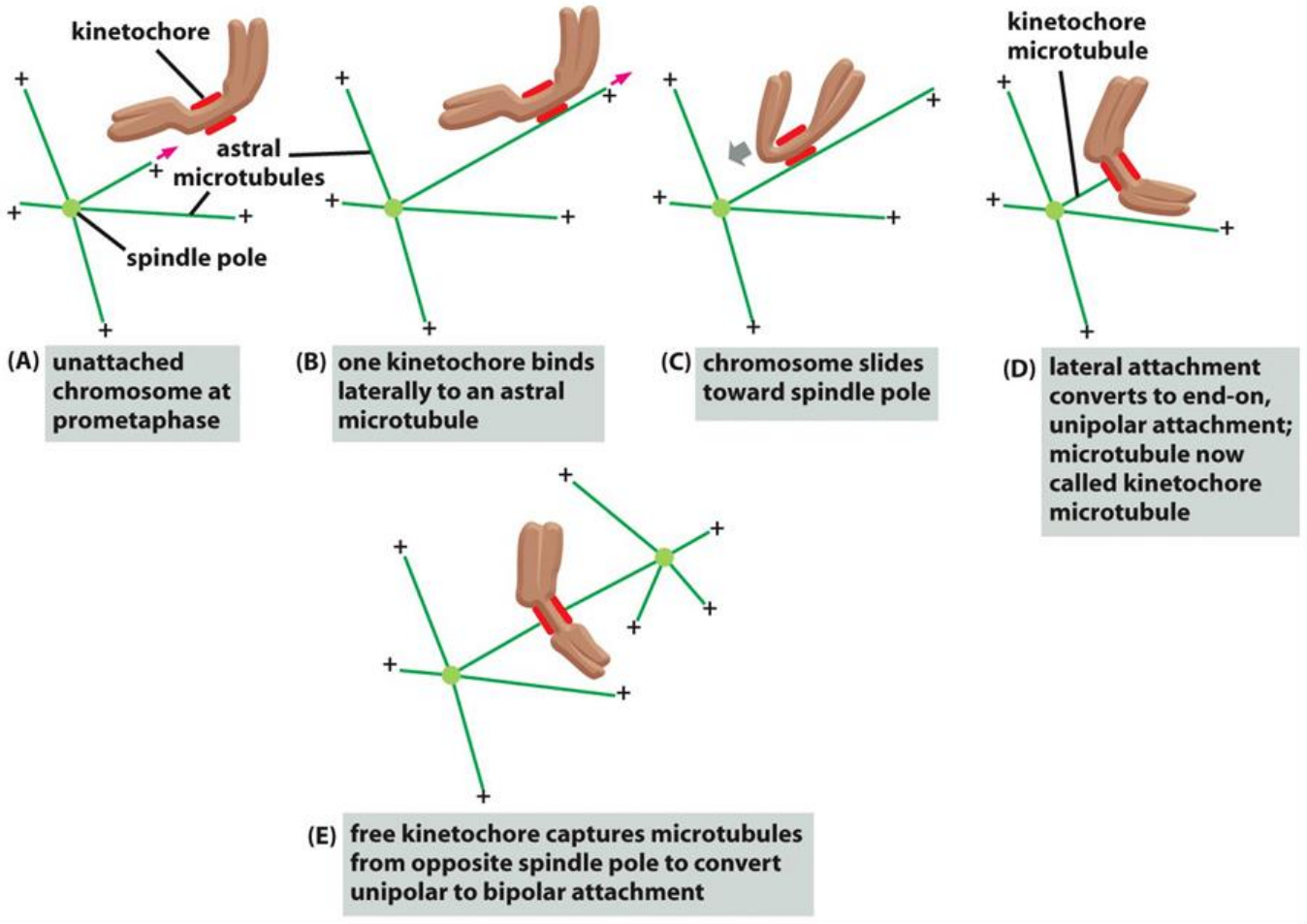
Kinetochore: multilayer protein structure built on the heterochromatin in the centromere.



Minus end

Plus end, the growing end

The capture of centrosome microtubules by kinetochores

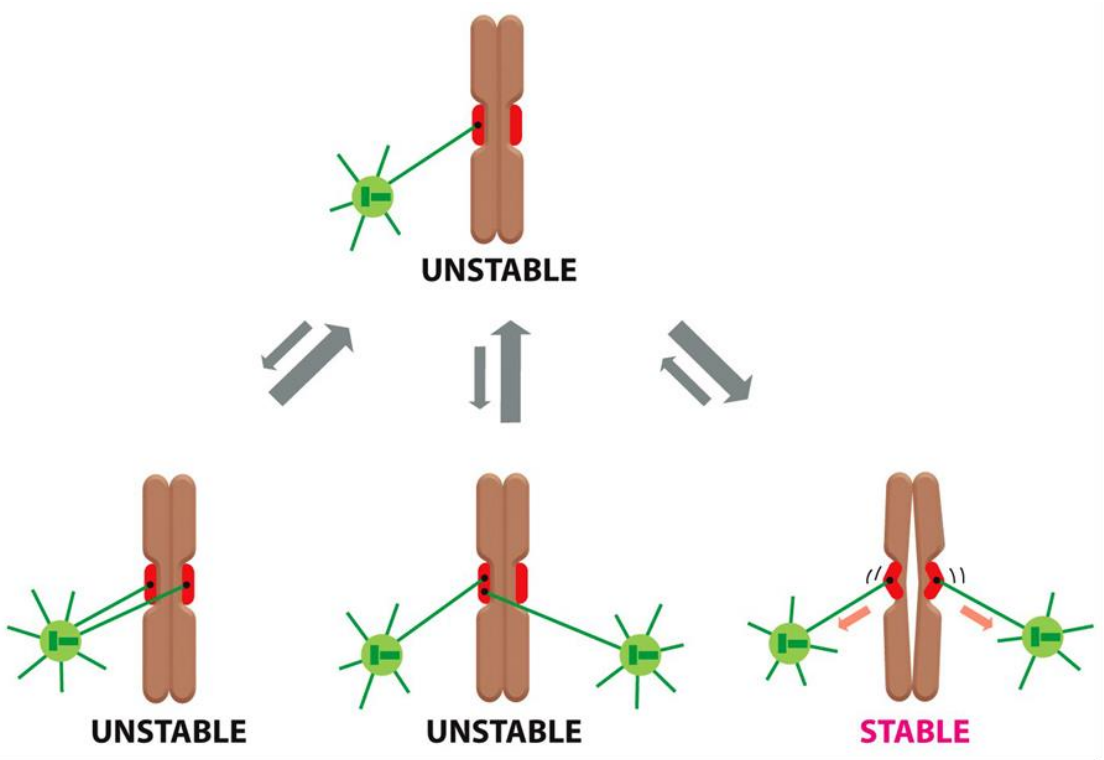


What about acentrosomal kinetochore attachment to the microtubules?

- Firstly, short microtubules close to chromosomes interact with kinetochores
- Secondly, growth of the embedded plus ends result in growth of the microtubules away from the kinetochore.
- Thirdly, motor proteins crosslinks and focus the minus ends in the spindle poles.

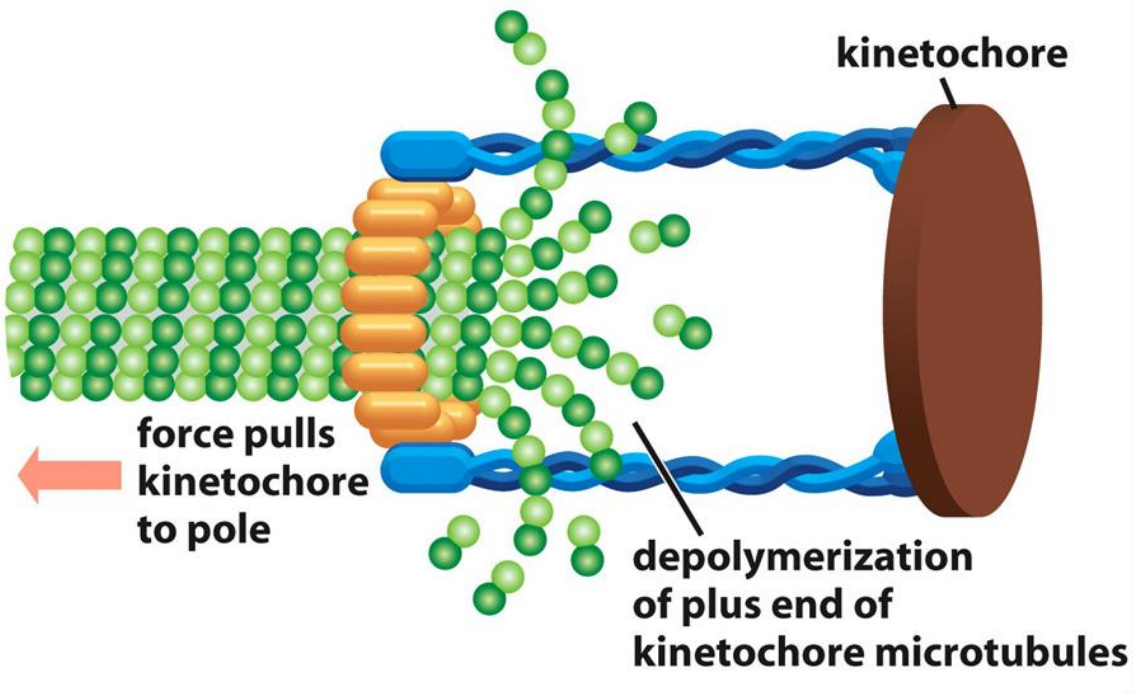
How to eliminate errors in kinetochore attachment in the sister chromatids?

- A tension sensing mechanisms through protein kinase aurora B

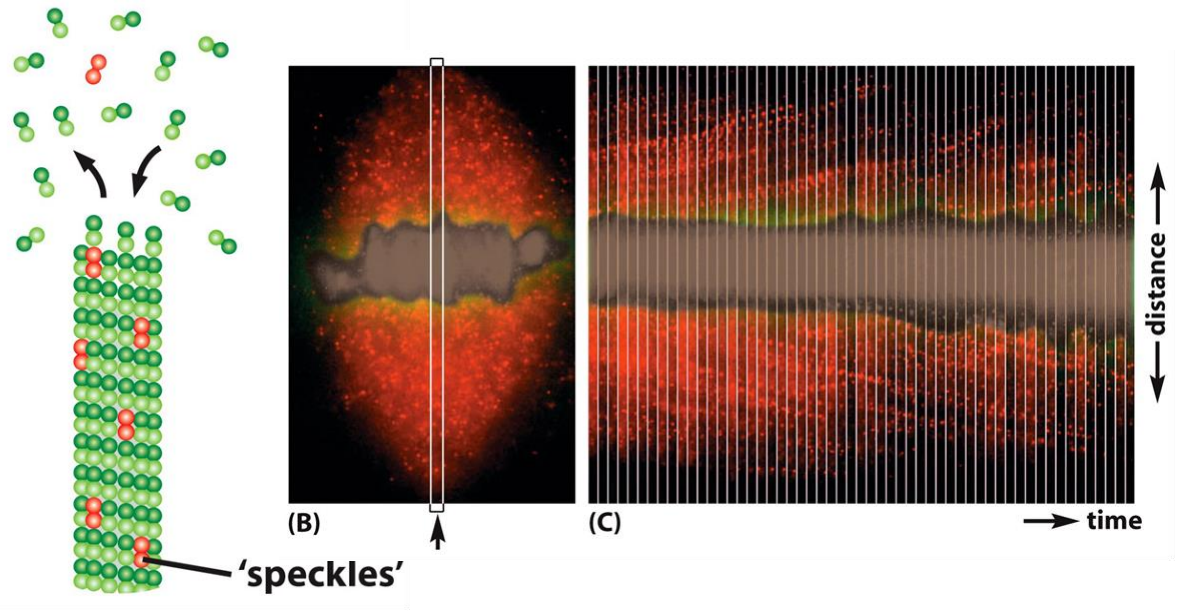


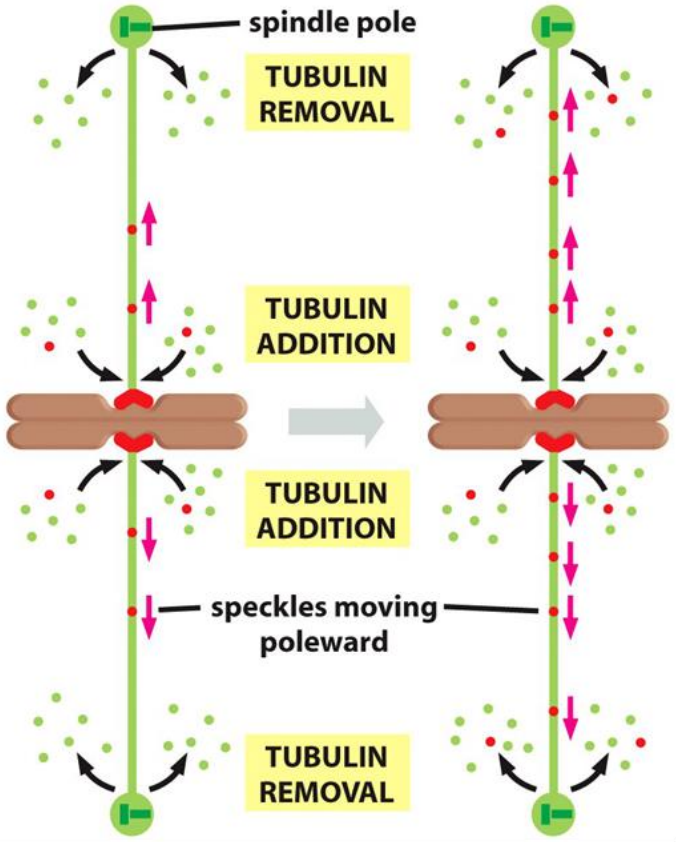
Three major forces move chromosomes on the spindles

- 1. Depolymerization at the plus end of the microtubule pull kinetochores.

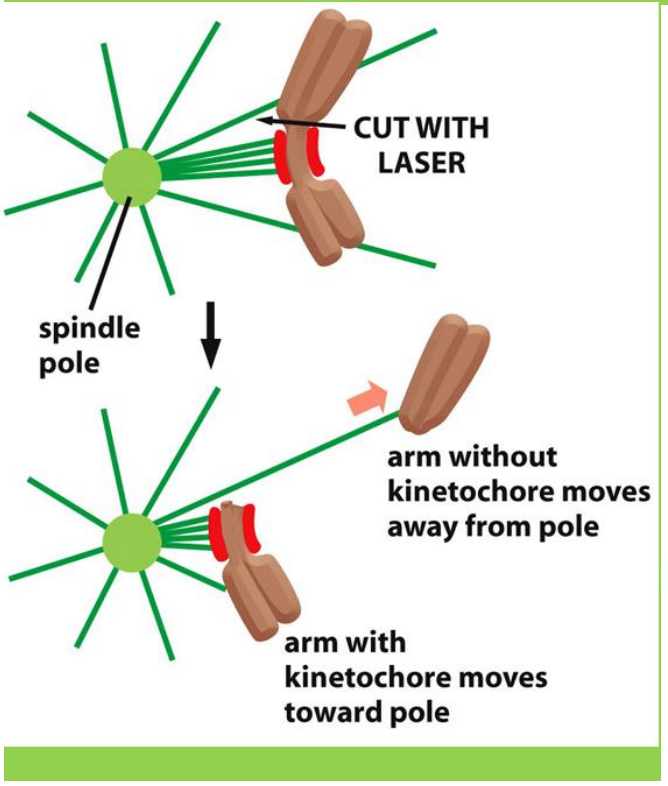


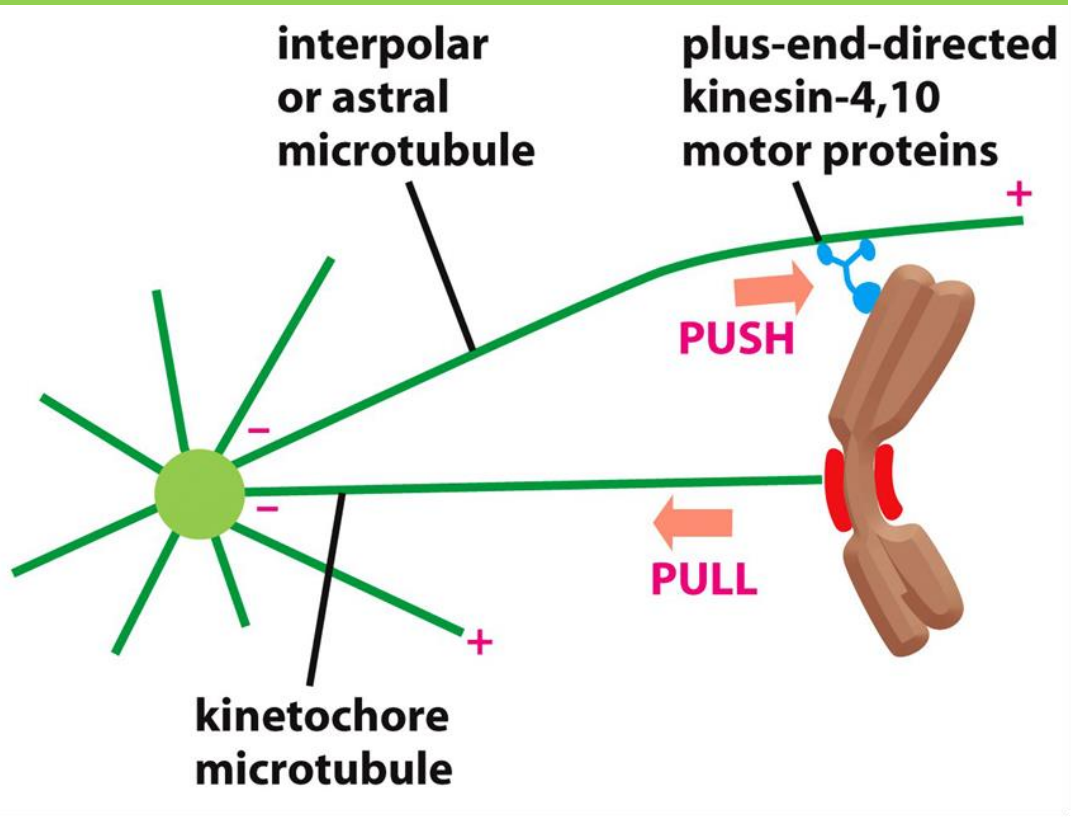
- 2. microtubule flux in the metaphase spindle





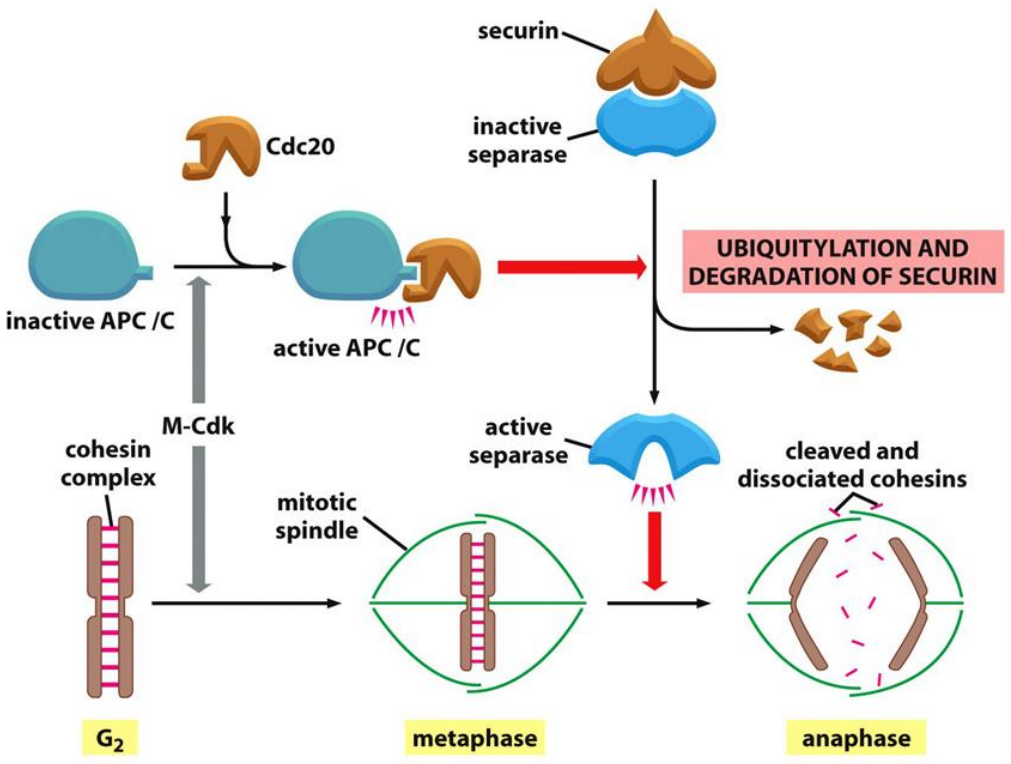
- 3. polar ejection force





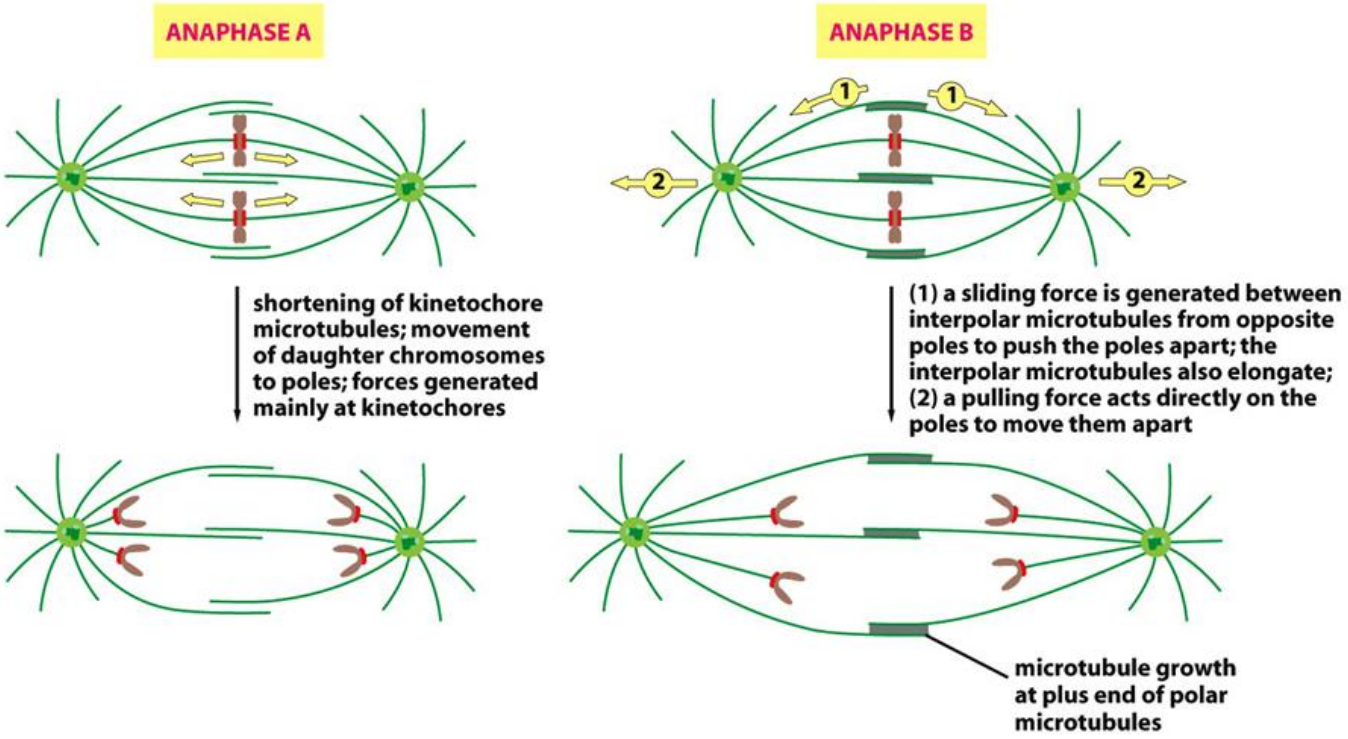
APC/C: metaphase-to-anaphase transition

- Degrade securin, release of separase, which cleaves cohesins on sister chromatids.
- Degrade S-cyclin and M-cyclins



Chromosome segregate in anaphase

- Anaphase A: initial stage, shortening of the kinetochore microtubules
- Anaphase B: later stage, separation of the spindle poles.



Telophase

- Disassembly of the mitotic spindle
- Re-formation of the nuclear envelope (fragments associated with chromosomes, they coalesce to re-form the complete nuclear membrane)
- Dephosphorylation on important effectors plays roles in causing these effects.

V. Cytokinesis (division of cytoplasm)

- Overview of cytokinesis

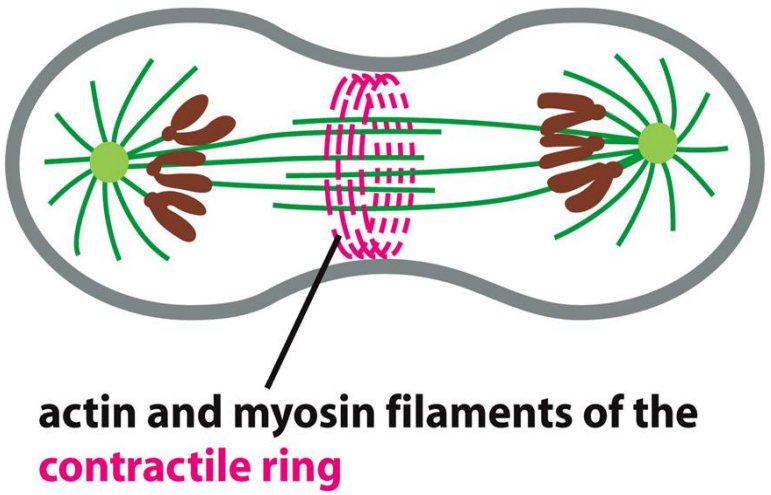
  four stages: initiation: contractile ring formation

  contraction: myosin II/actin contraction

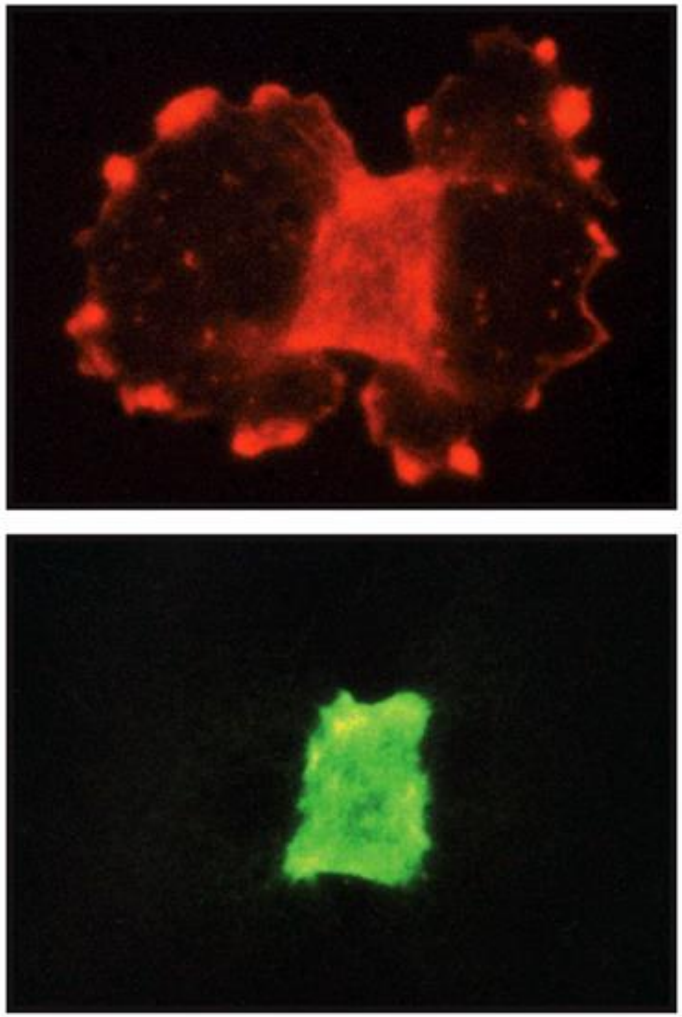
  membrane insertion: plasma membrane addition

  completion

17.5-interpret_mitosis.mov



The contractile ring

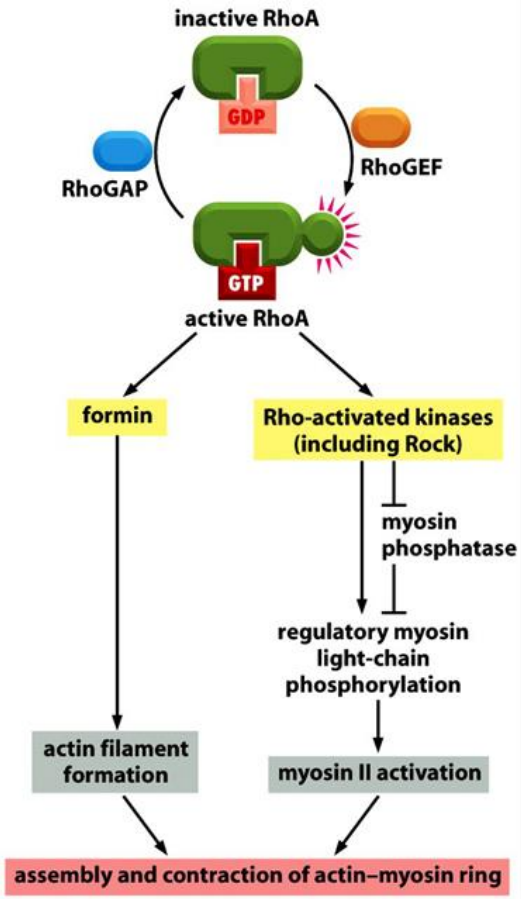


Actin in dividing cell

Myosin II in the same dividing cell

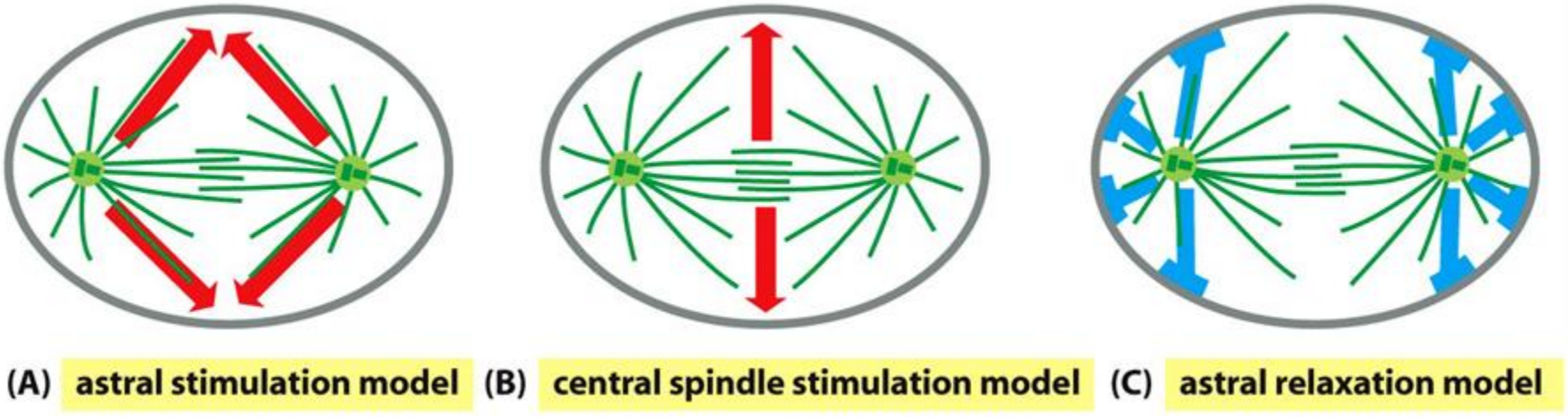
How is the contractile ring formed?

- Rho GEF activates Rho-GTP
- Rho-GTP activates formin which assemble actin filaments, Rock which activates regulatory Myosin light chains, triggering contraction.



Spindles determine the position of the cleavage furrow

- Three models have been proposed

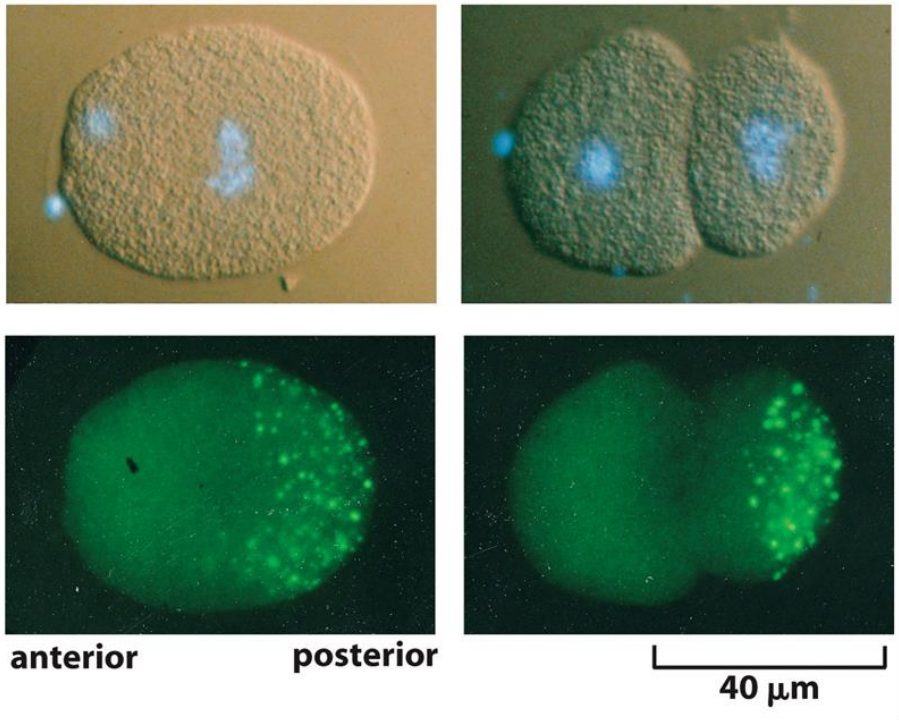


Organelle division

- Organelle grow from pre-existing organelle and double in cell cycle.
- Mitochondria and chloroplasts double and divide in each cell cycle
- ER remain intact, organized by microtubule, and cut into two halves in cytokinesis.
- Golgi apparatus fragmented and reorganized. associated with spindle poles.

Symmetrical and asymmetrical division

- Most are symmetrical
- During development, asymmetrical division is associate with different fate in cell development, the spindle positioning is important and nicely controlled



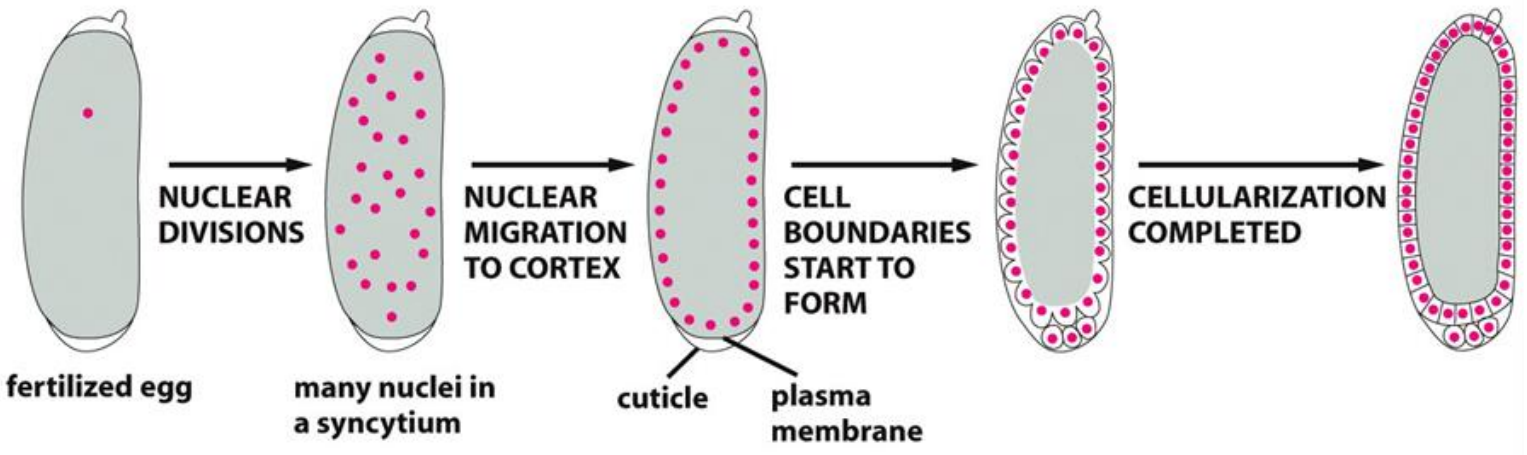
Blue -nucleus

Green: P-granule(RNA, protein) decides germ cells formation

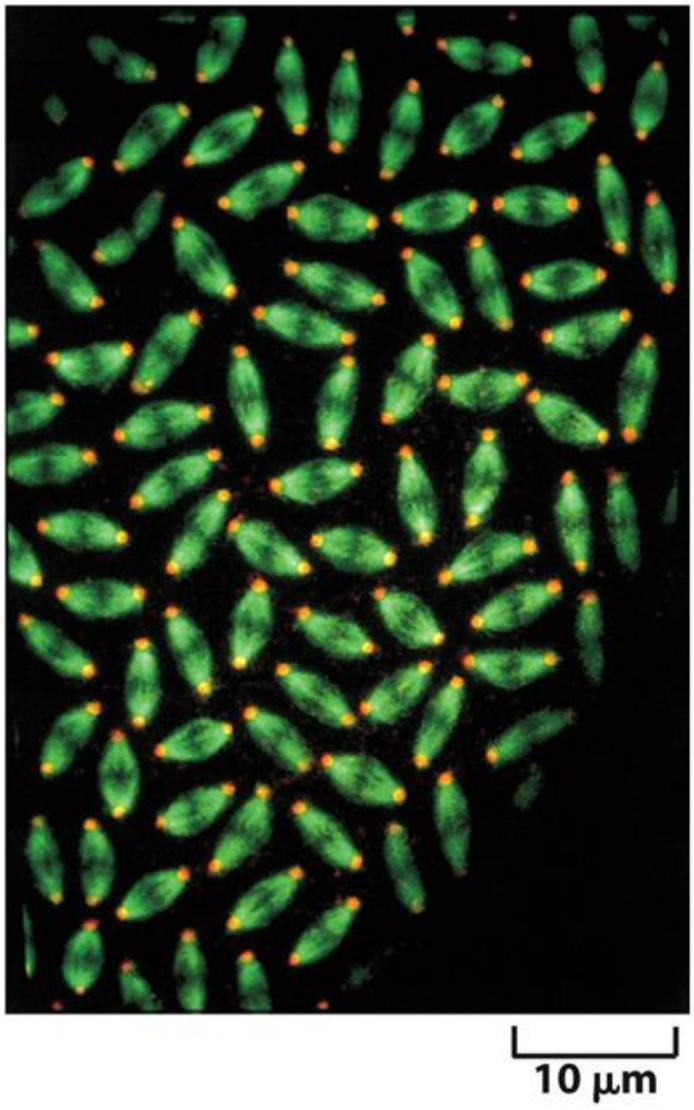
Mitosis without cytokinesis

- Drosophilae embryo
- Megakaryotcytes---blood platelets
- Hepatocytes
- Heart muscle cell

syncytium and cellularization for Drosophila embryo



An image for synchronized nuclei in Drosophila embryo



VI. Control of cell division and cell growth

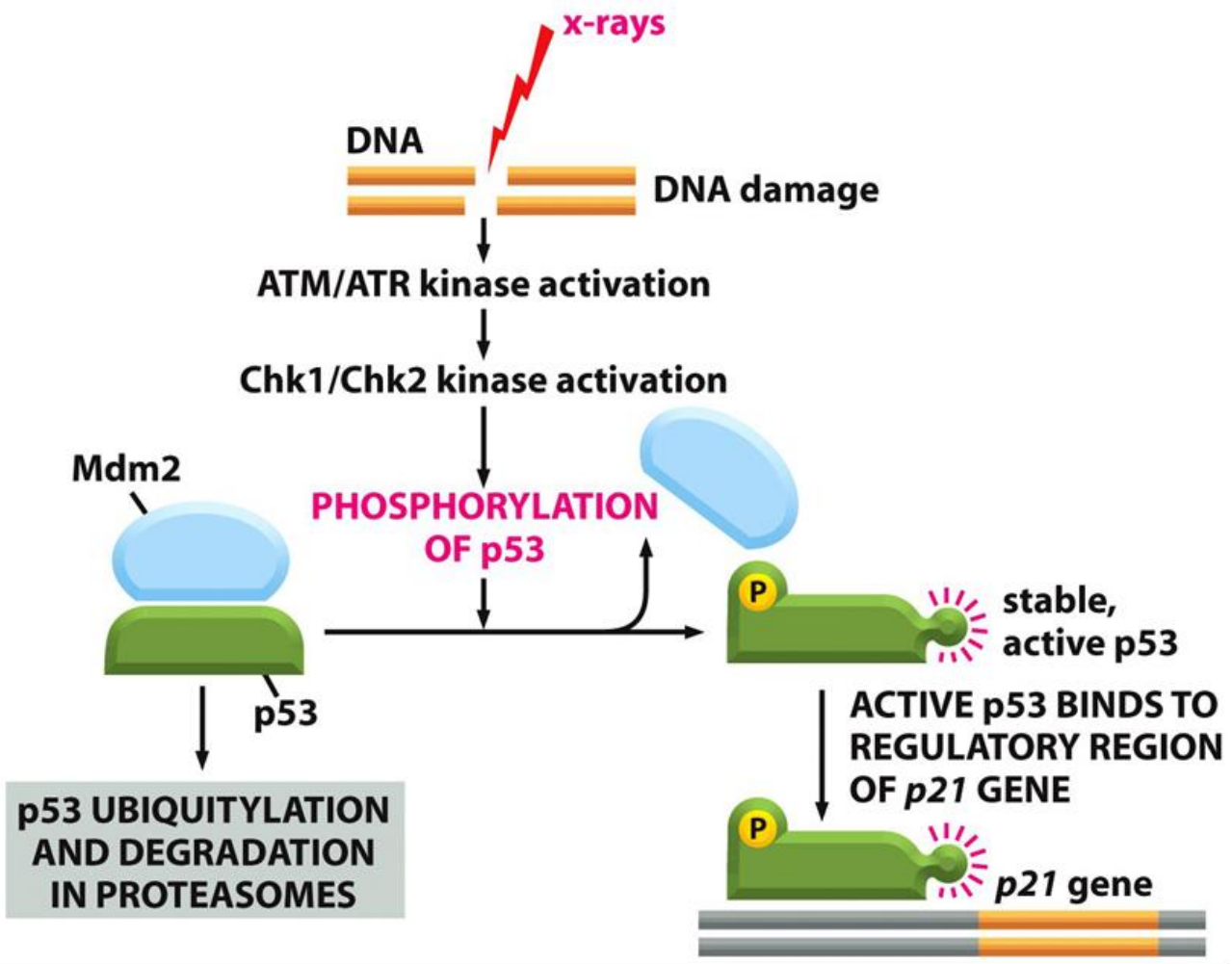
- 1. Overview
- 2. DNA damage blocks cell division
- 3. Mitogens stimulate cell division
- 4. Coordination between growth and division

1. Overview

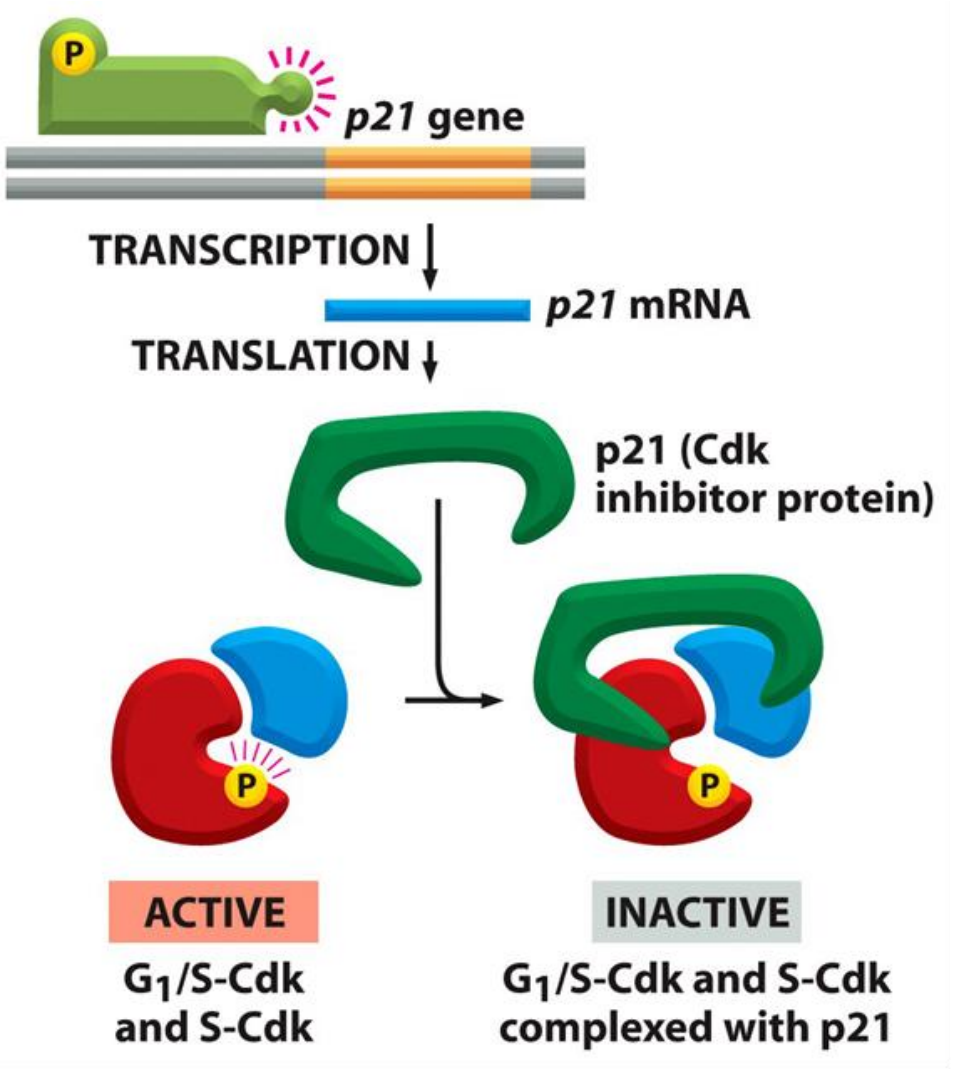
- What is controlling organ size and organism size?

1. Mitogens stimulate cell division
2. Growth factors stimulate cell growth (increase in cell mass)
3. Survival factors prevents cell death.

2. DNA damage response



Arrest cell cycle both at G1/S and G2/M Transition.



Defects in DNA damage response

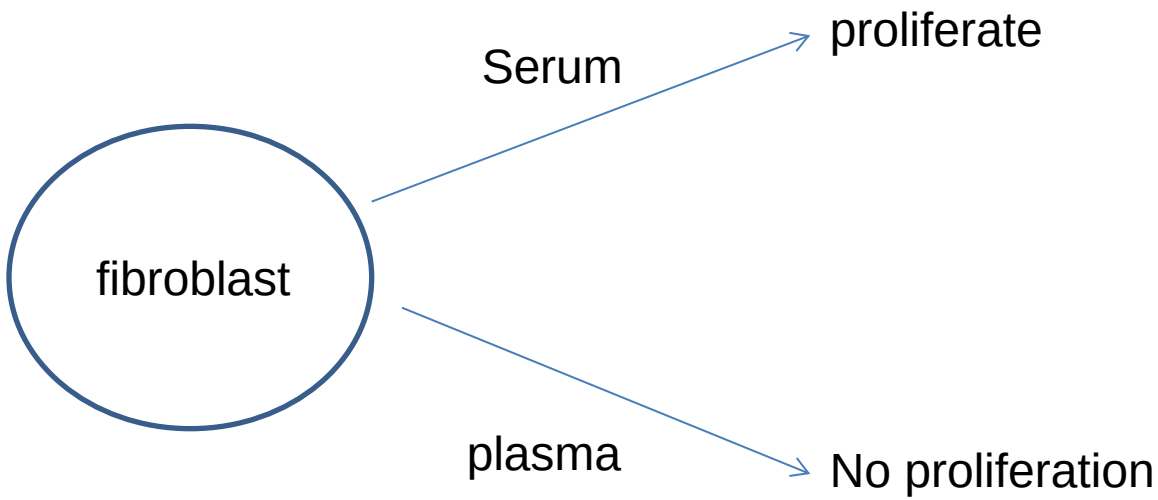
- ATM mutation : ataxia telangiestasia

  serious genetic disease, hypersensitive to sunlight, few live beyond 20s, neurodegenerated,

  Highly susceptibility to cancer.

- P53 mutation/loss: cancer, ~50% of human cancer have p53 loss or mutation

3. Mitogens stimulate cell division



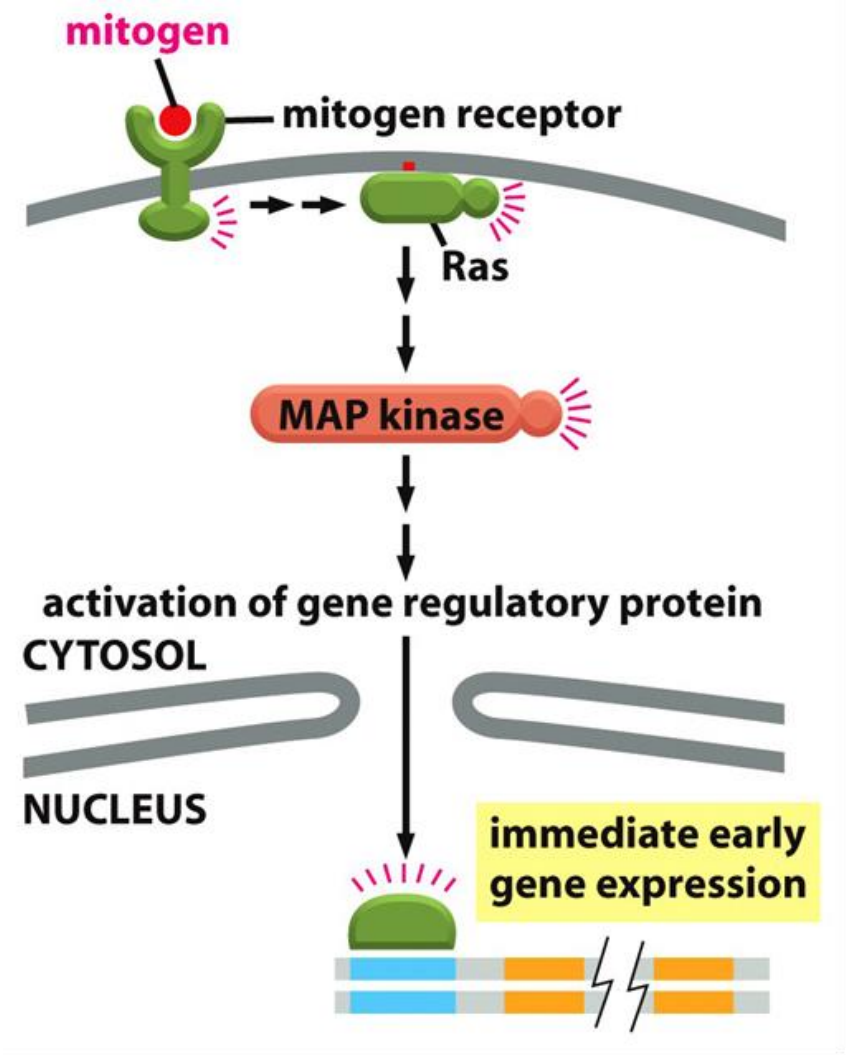
Difference between serum and plasma? Both derive from blood without cells, but Serum is supernatant after blood clotting, plasma is supernatant prior to blood clotting

Platelet during blood clotting release PDGF

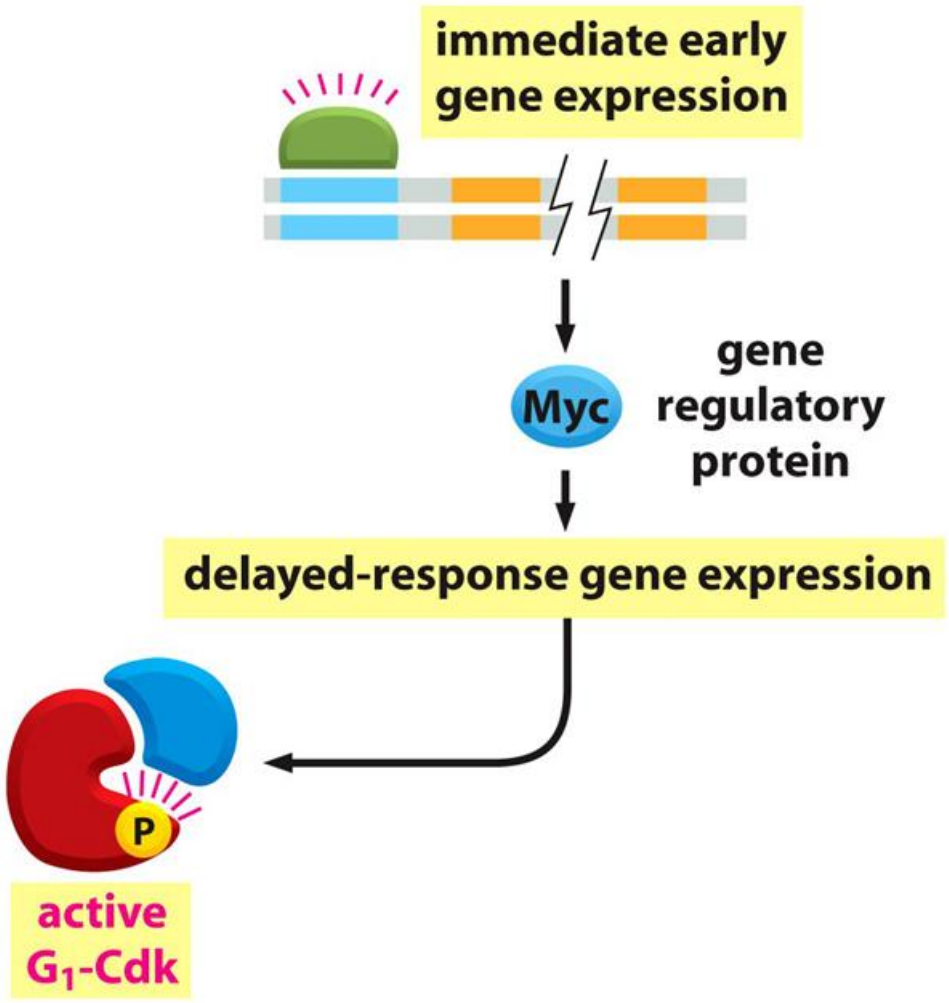
Mitogens

- Over 50 different mitogens, including EGF, FGF, NGF, erythropoietin, etc.
- Functions by triggering a wave of G1/S –Cdk activity that relieves intracellular negative controls.
- Mainly through phosphorylation on Retinoblastoma (Rb) protein, liberating E2F proteins, which acts as transcription factors to simulate G1/S cyclins, etc.

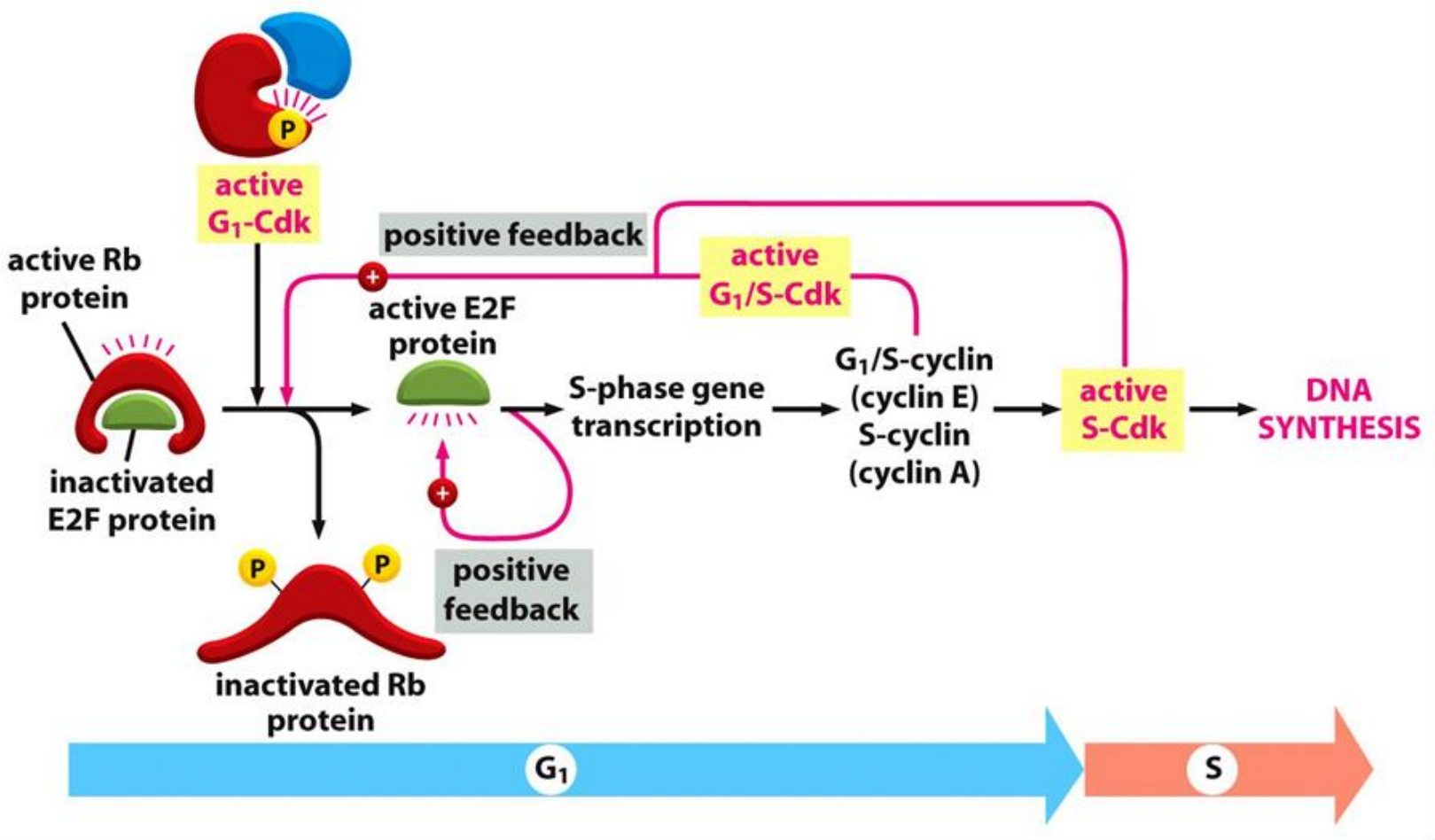
The signaling pathway controlling cell cycle progression



MAP transcriptionally activates c-Myc

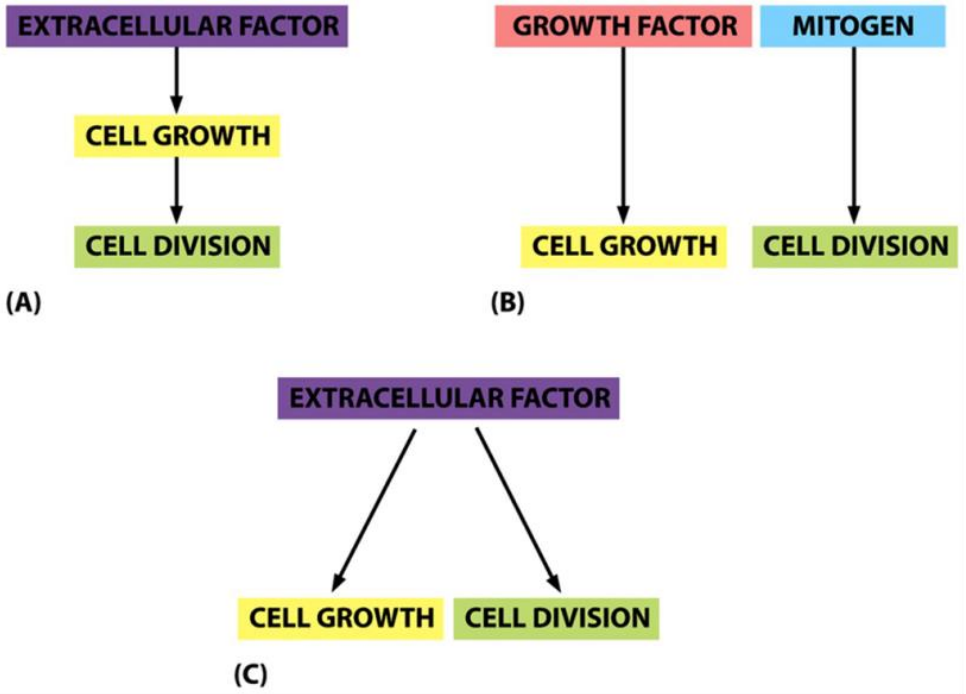


Rb-E2F pathway in cell cycle control



4. Coordination of cell growth and cell division

- Not always coupled: terminally differentiated cells such as muscle and nerve cells growth but no division; drosophilae fertilized eggs division without growth.
- Several coordination mechanisms exist



Many questions remain

- How is cell size controlled precisely?
- How is cell division numbers control precisely?
- How to maintain the homeostasis in an organism?