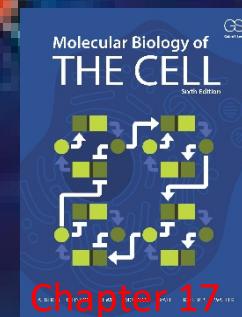


## Lecture 15

# The cell cycle II

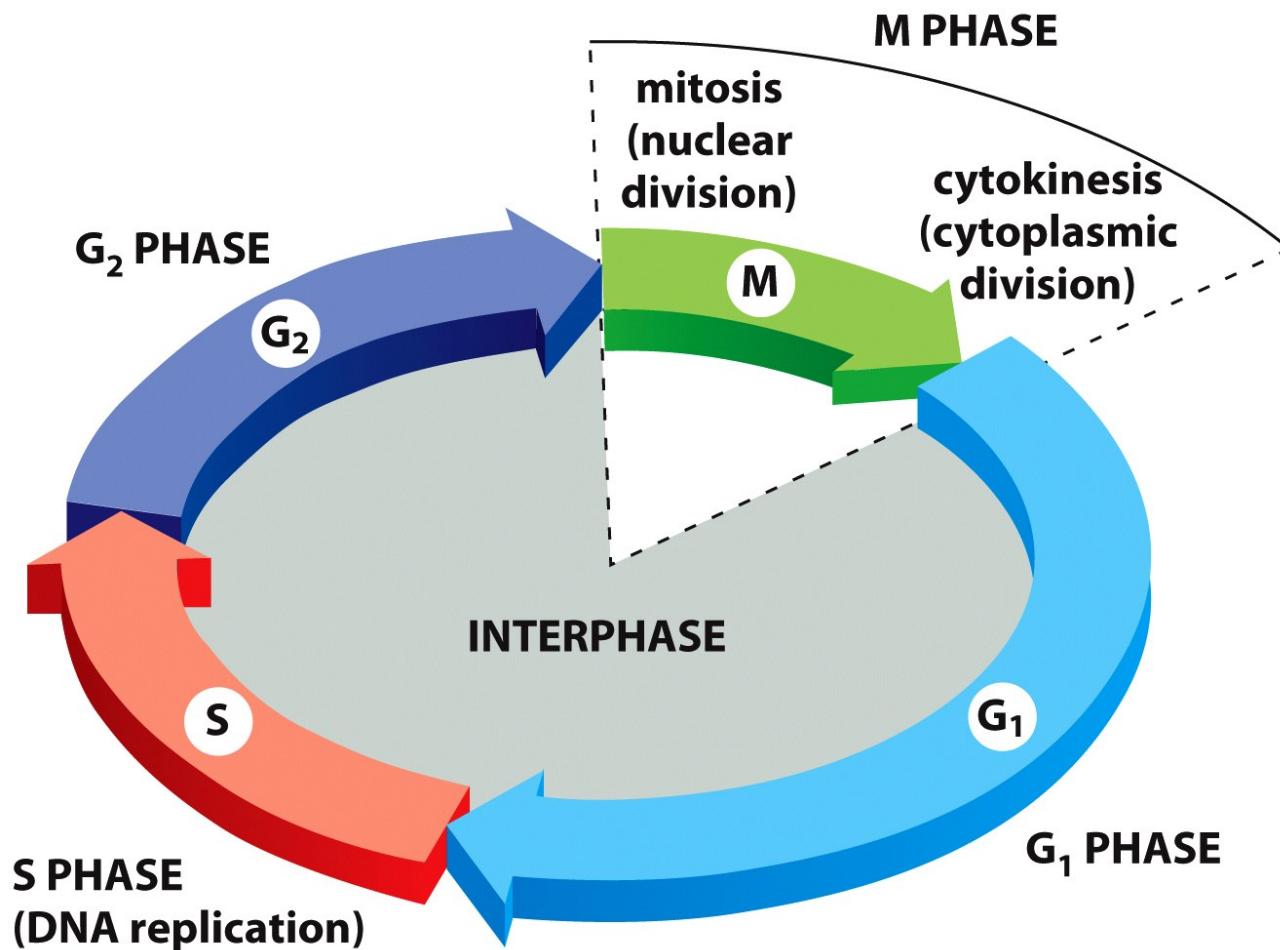
### Outline

- I. M-phase: Mitosis
- II. M-phase: Cytokinesis
- III. Control of cell division and cell growth



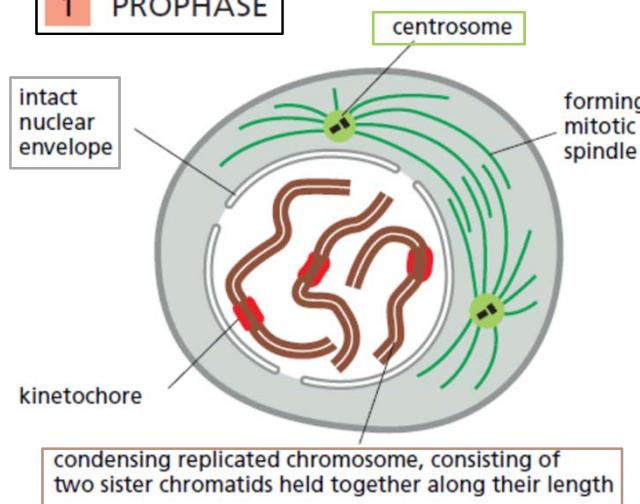
Chapter 17

## The cell cycle: remember last week?

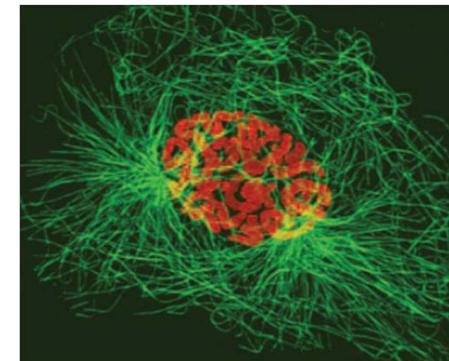


# The M-phase: Mitosis - What we can see...

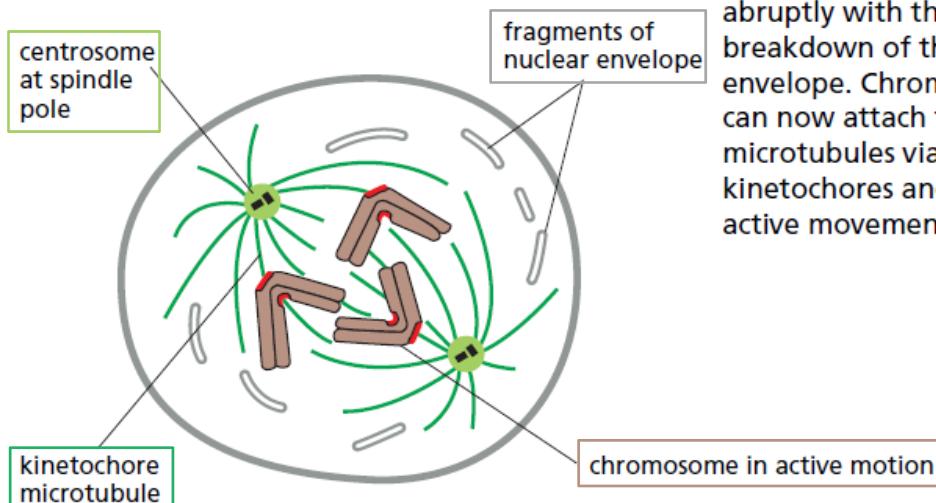
## 1 PROPHASE



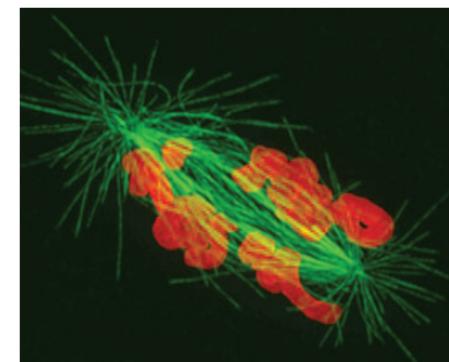
At **prophase**, the replicated chromosomes, each consisting of two closely associated sister chromatids, condense. Outside the nucleus, the mitotic spindle assembles between the two centrosomes, which have replicated and moved apart. For simplicity, only three chromosomes are shown. In diploid cells,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



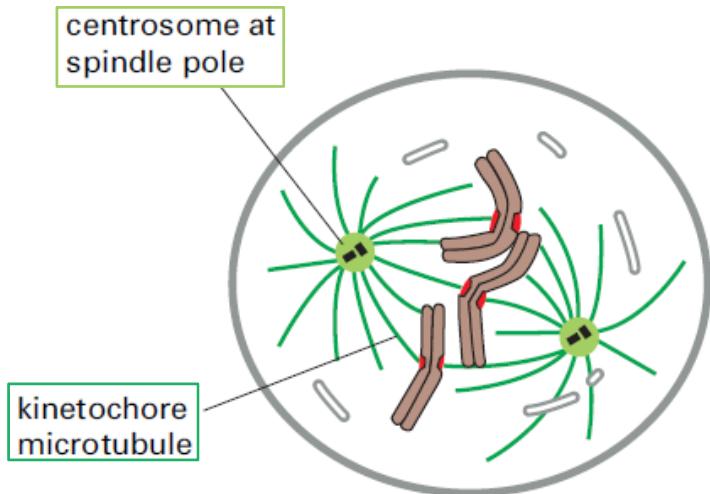
## 2 PROMETAPHASE



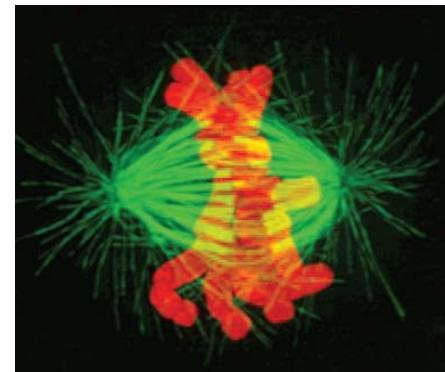
**Prometaphase** starts abruptly with the breakdown of the nuclear envelope. Chromosomes can now attach to spindle microtubules via their kinetochores and undergo active movement.



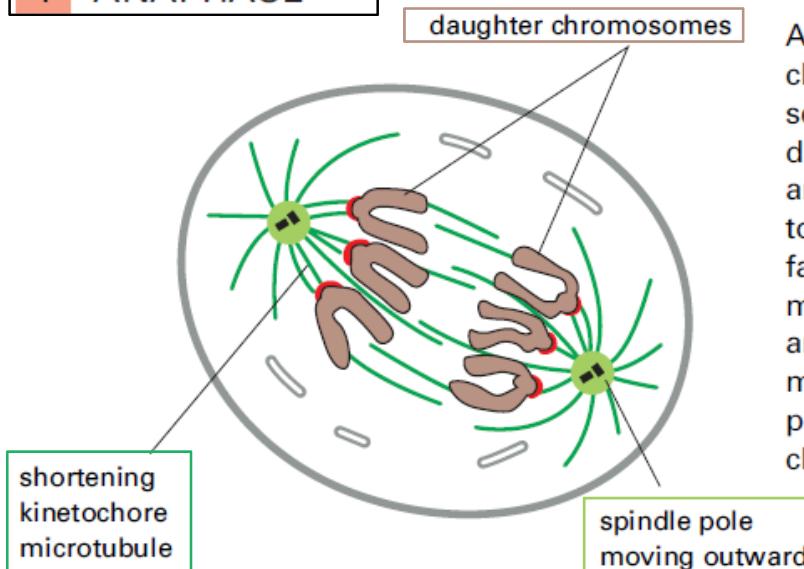
### 3 METAPHASE



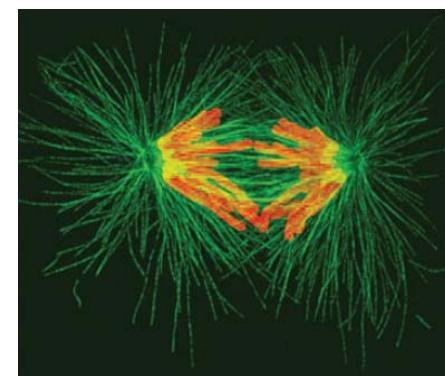
At **metaphase**, the chromosomes are aligned at the equator of the spindle, midway between the spindle poles. The kinetochore microtubules attach sister chromatids to opposite poles of the spindle.



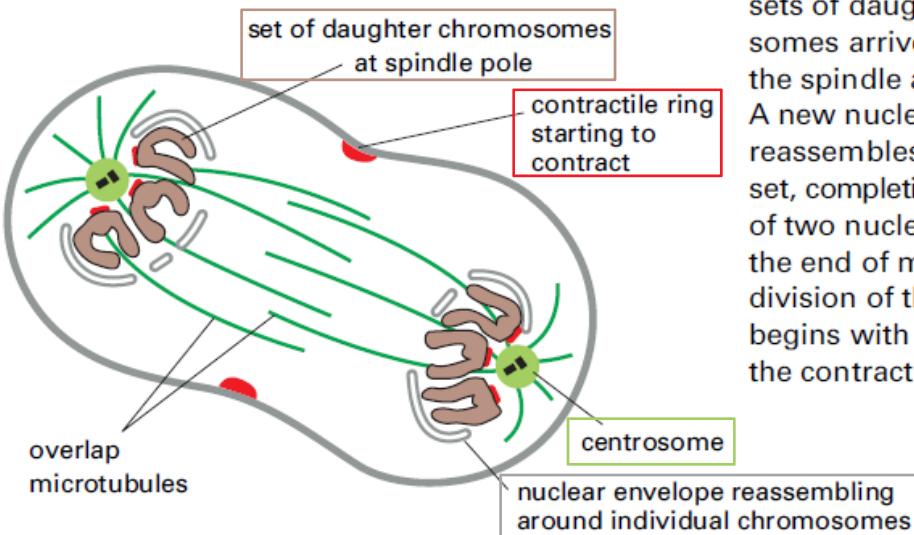
### 4 ANAPHASE



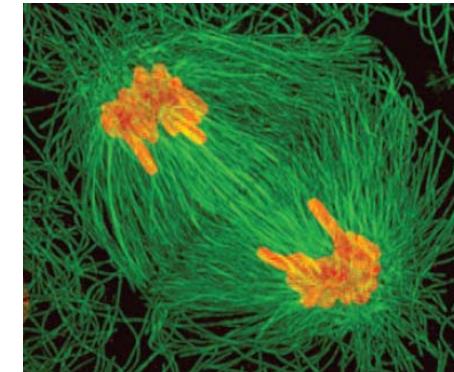
At **anaphase**, the sister chromatids synchronously separate to form two daughter chromosomes, and each is pulled slowly toward the spindle pole it faces. The kinetochore microtubules get shorter, and the spindle poles also move apart; both processes contribute to chromosome segregation.



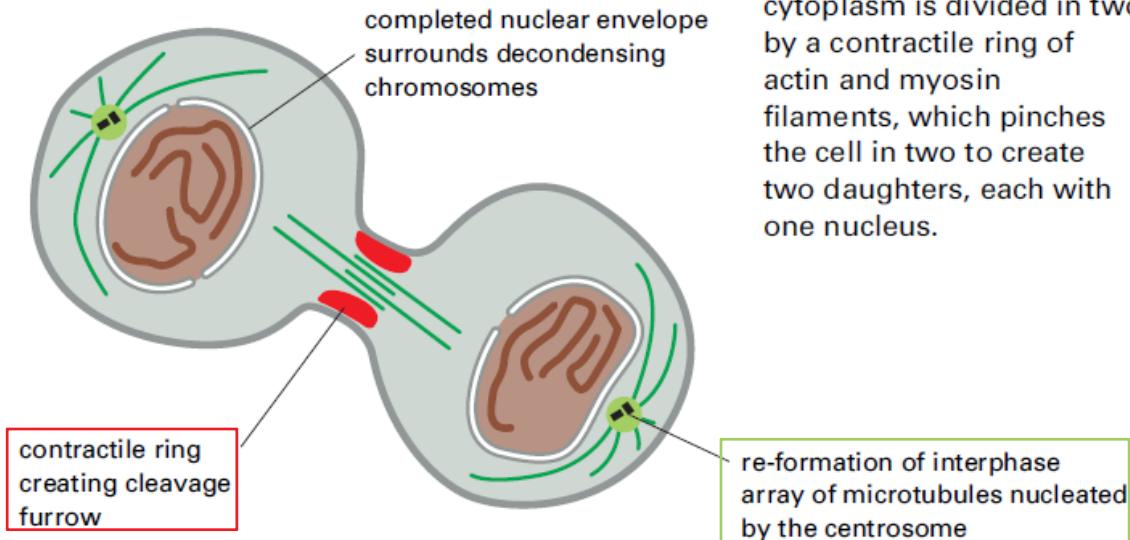
## 5 TELOPHASE



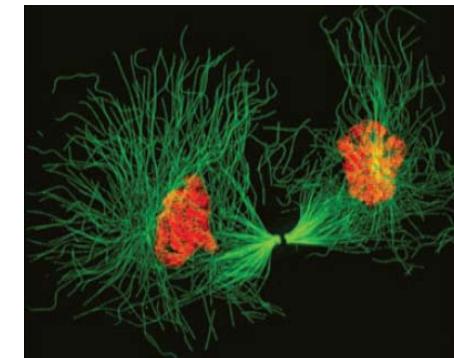
During **telophase**, the two sets of daughter chromosomes arrive at the poles of the spindle and decondense. A new nuclear envelope reassembles around each set, completing the formation of two nuclei and marking the end of mitosis. The division of the cytoplasm begins with contraction of the contractile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



## Mitosis can be divided into two phases

### Phase 1: From prophase via prometaphase to metaphase:

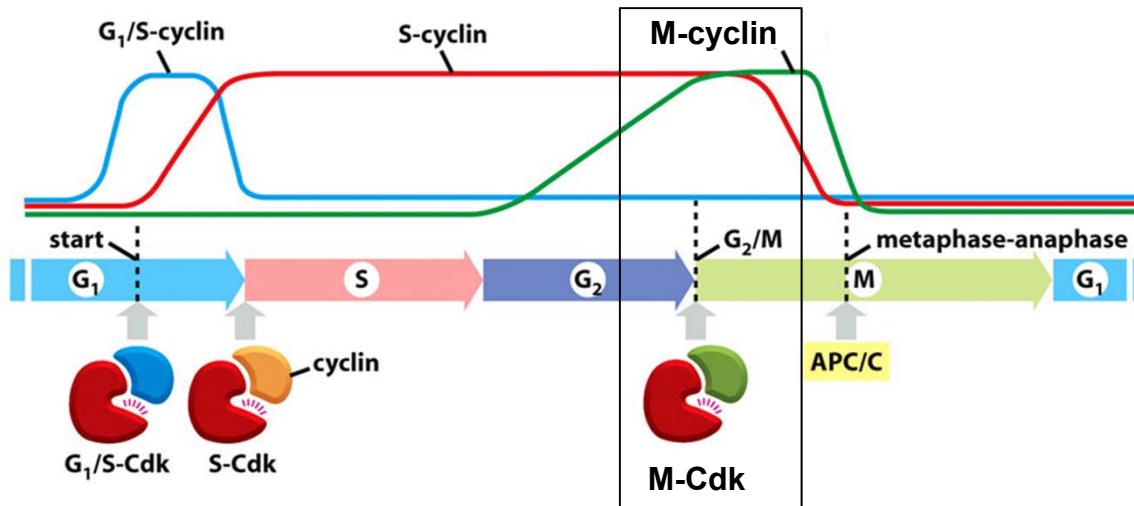
- **M-Cdk (M-cyclin-dependent kinase)** and several other mitotic protein kinases **phosphorylate** a variety of **proteins**  
→ triggers assembly of the mitotic spindle  
& attachment of the sister chromatid pairs

### Phase 2: From metaphase to anaphase transition:

- **APC/C (anaphase-promoting-complex / cyclosome)**  
→ triggers the cleavage of cohesions  
& initiates segregation of sister chromatids.
- APC/C also triggers destruction of cyclins to deactivate Cdks.
- APC/C also triggers finally the disassembly of the mitotic spindle.

# Early events in Mitosis: M-Cdk drives entry into mitosis

Remember:



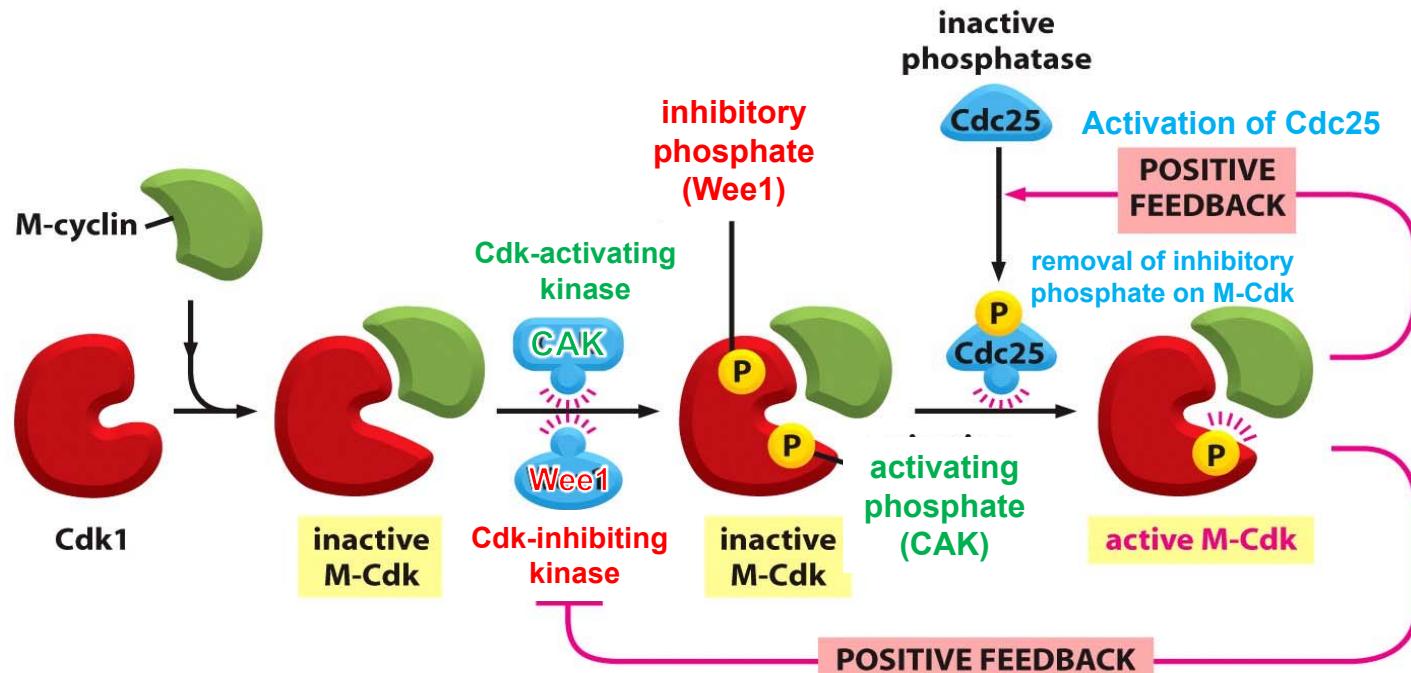
## M-Cdk (complex of Cdk1 and M-cyclin) and its effectors:

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 the Golgi

**How?** Not fully understood; not all substrates for M-Cdk have been identified yet!

# Onset of mitosis: activation of the M-Cdk complex

M-Cdk it is finally activated by the phosphatase Cdc25.

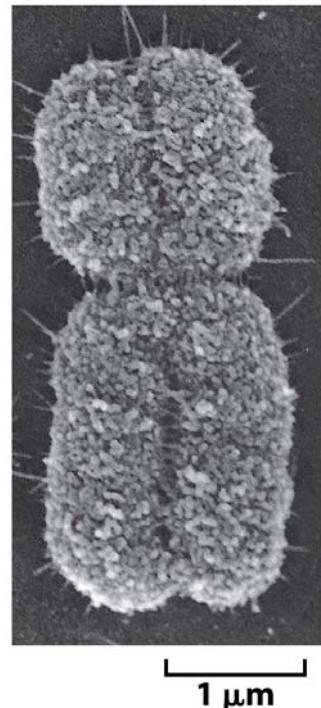


- Assembly of the complex from Cdk1 and M-cyclin (**inactive!**)
- Phosphorylation of **inhibitory & activating** sites by **Wee1 & CAK** (**complex remains inactive!**)
- Activation of the phosphatase Cdc25
- Active Cdc25 **activates** M-Cdk by **removing** the **inhibitory phosphate** (**positive feedback**)
- **Simultaneous inhibition** of the Cdk-inhibitory kinase Wee1 (**positive feedback**)

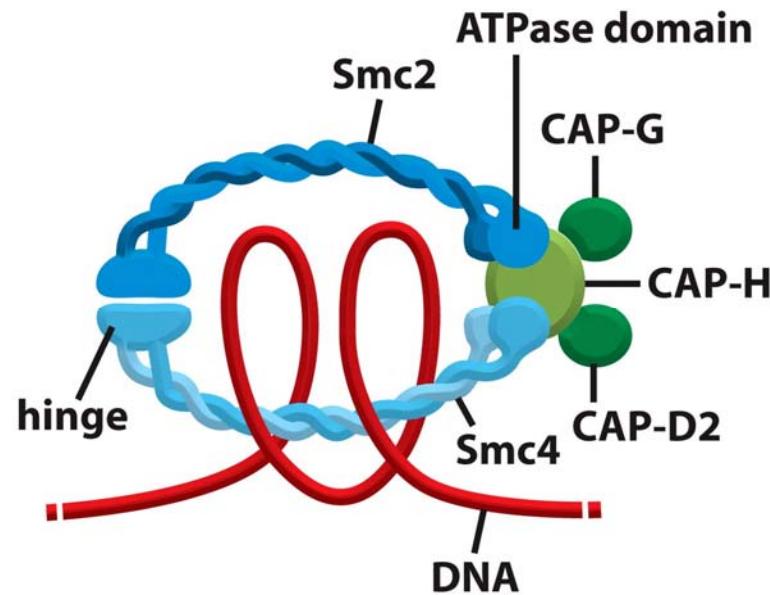
## Sister chromatin condensation by condensins: phosphorylated and activated by M-Cdk

**The mitotic chromosome:**

- **Two sister chromatids** joined along their length.
- The constricted regions hint to the centromere



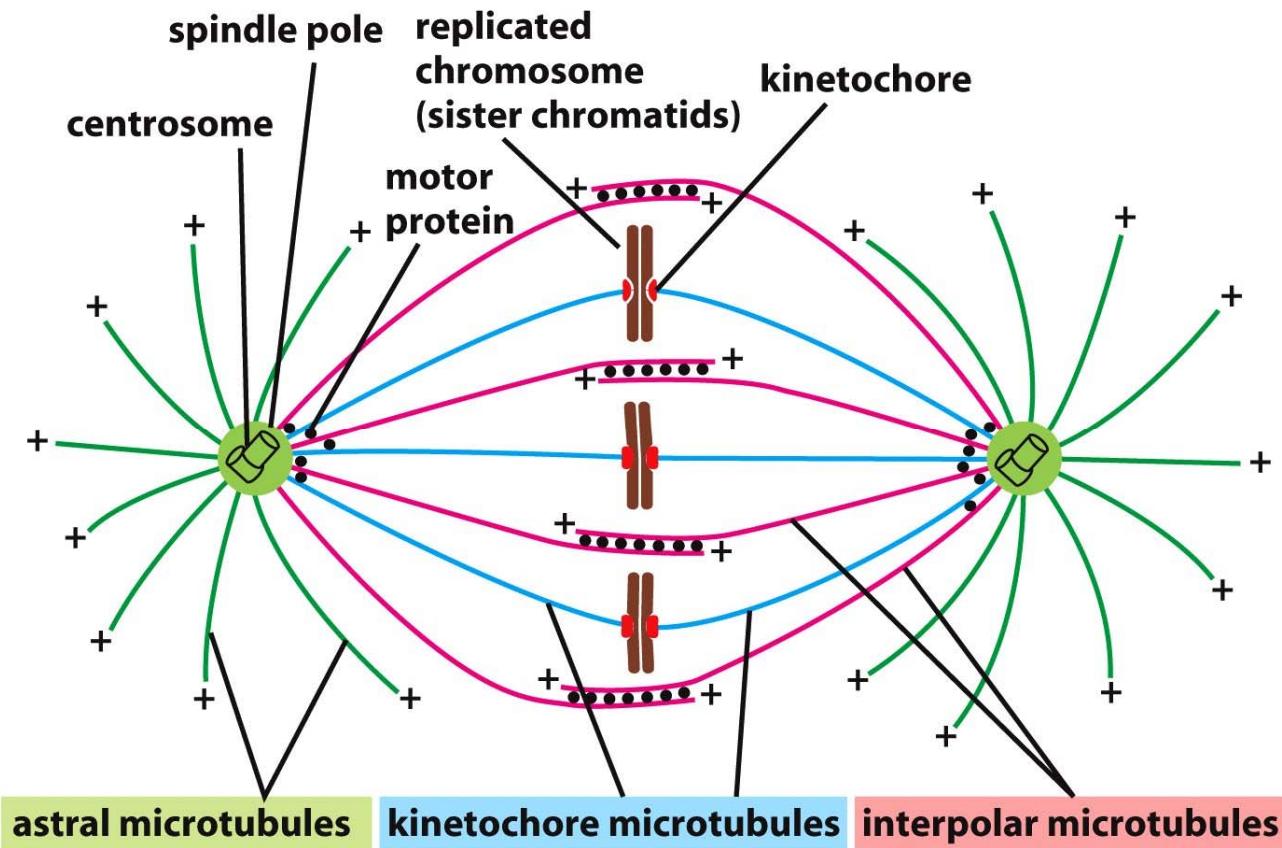
Condensin might form a ring-like structure and ATP hydrolysis to drive condensation...



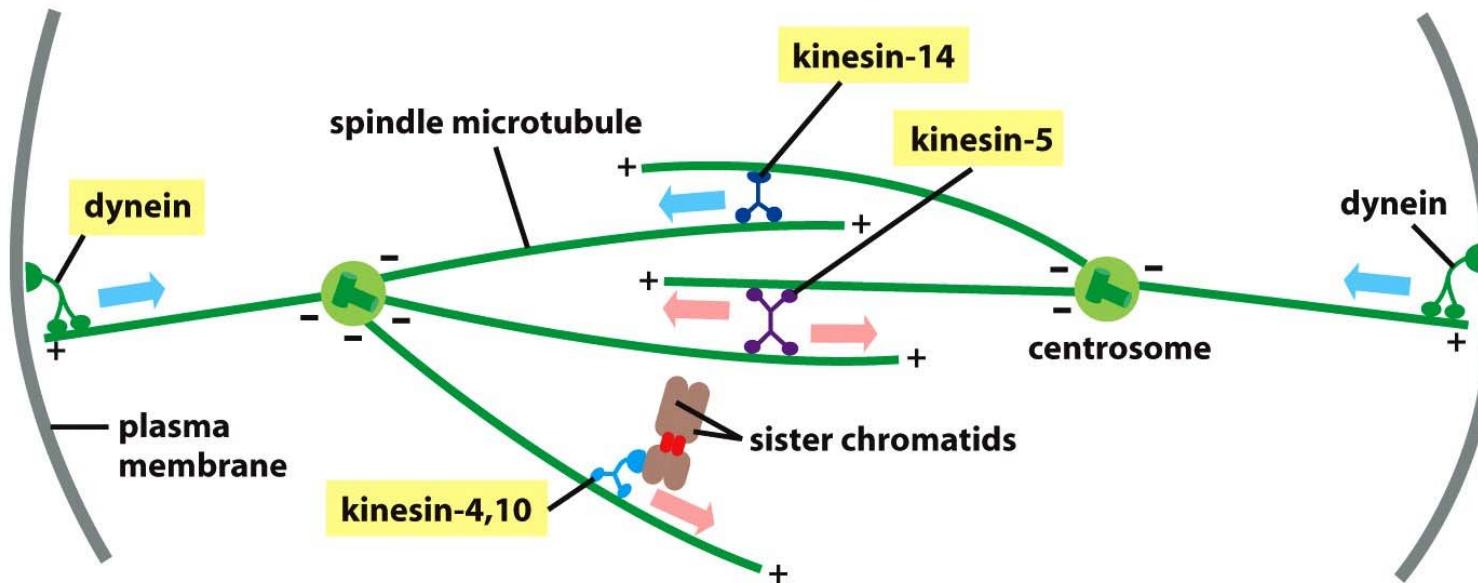
It is not clear how condensing restructures and compacts the chromosome DNA:  
It may form a ring structure that encircles loops of DNA within each sister chromatid....

M-Cdk also triggers formation of mitotic spindle

Three classes of microtubules:



# Microtubule-dependent motor proteins govern spindle assembly and function



- **Kinesin-5:** **pushes** the 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 the spindle apparatus

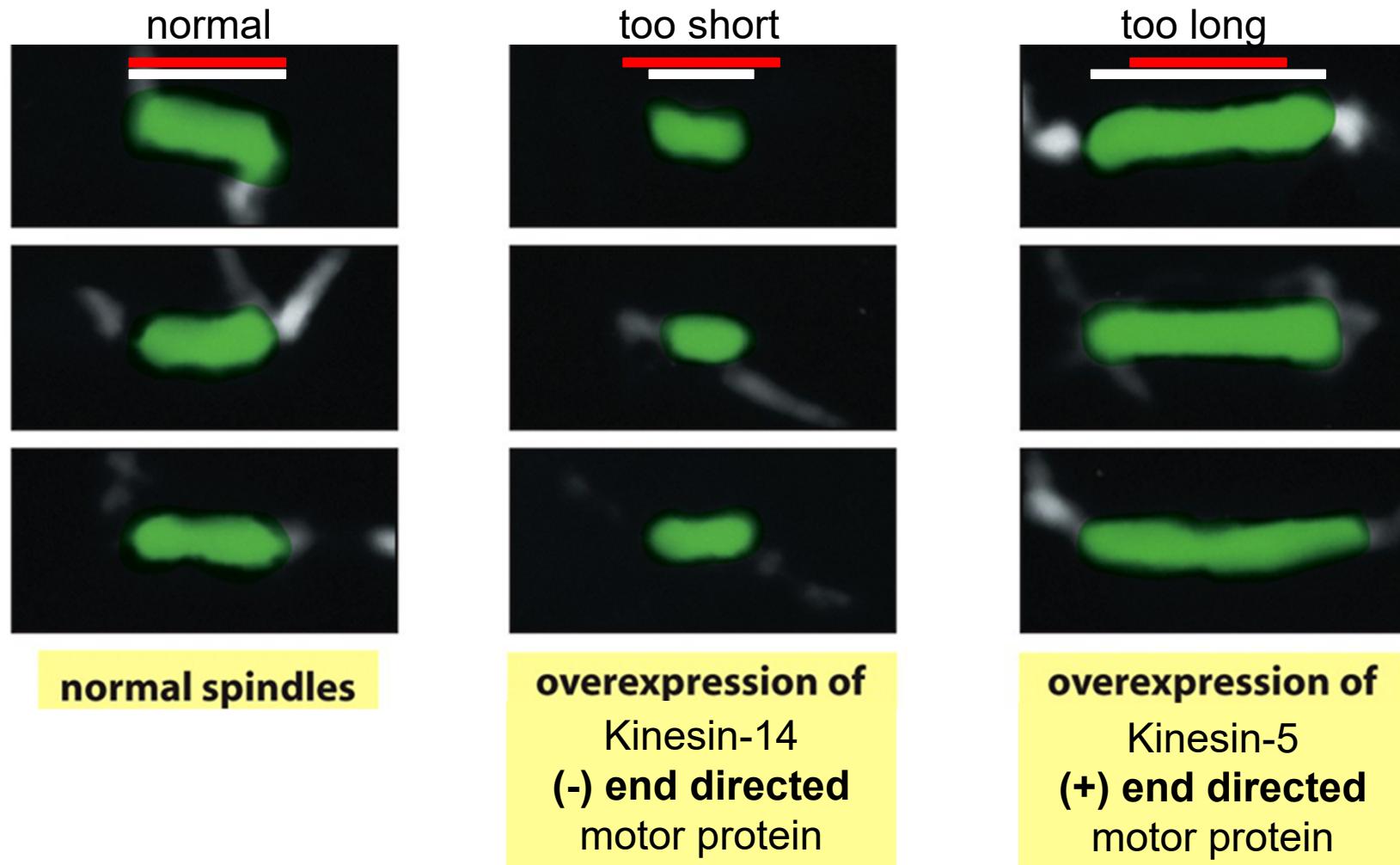
Two different mechanisms ensure bipolarity of spindles:

- A) Centrosome-dependent spindle formation
- B) Acentrosomal “self-organizing” of the spindle formation  
→Chromosome and motor proteins dependent

## A) Centrosome-dependent spindle assembly

- M-Cdk promotes **centrosome maturation**
  - amount of  $\gamma$ -tubulin ring complex ( $\gamma$ -YuRC ) increases  
→ more nucleating microtubules.
- M-Cdk (and other mitotic protein kinases e.g. Aurora-A) promote **centrosome separation**
- Motor proteins (kinesins and dynein) provide the mechanical energy to push the spindle poles apart
  - **kinesin-5**: **pushes** centrosomes **away** from each other
  - **dynein**: **pulls** centrosomes towards the PM (away from each other)
  - **kinesin-14** : pull centrosomes together
- All these activities **reach a balance** that determines the final length of spindles.  
(→mechano-sensing? Undiscovered lands?)

## Experimental evidence for the opposing effects of kinesin-14 and kinesin-5 on spindle length



## Attachment of sister-chromatid pairs to the spindle requires disassembly of the nuclear envelope

**M-Cdk** and other mitotic protein kinases **phosphorylate** components of the **nuclear pore complex** and **laminin**:

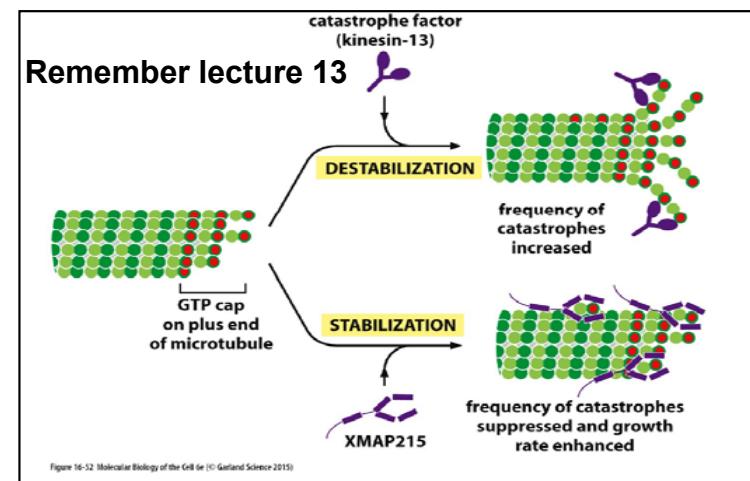
- This **promotes the disassembly** of the nuclear envelope and of the nuclear lamina (the structural compound of the envelope).
- The nuclear envelope **disintegrates** into vesicles and ER

In mitosis, microtubule arrangement changes drastically and the microtubule instability increases greatly

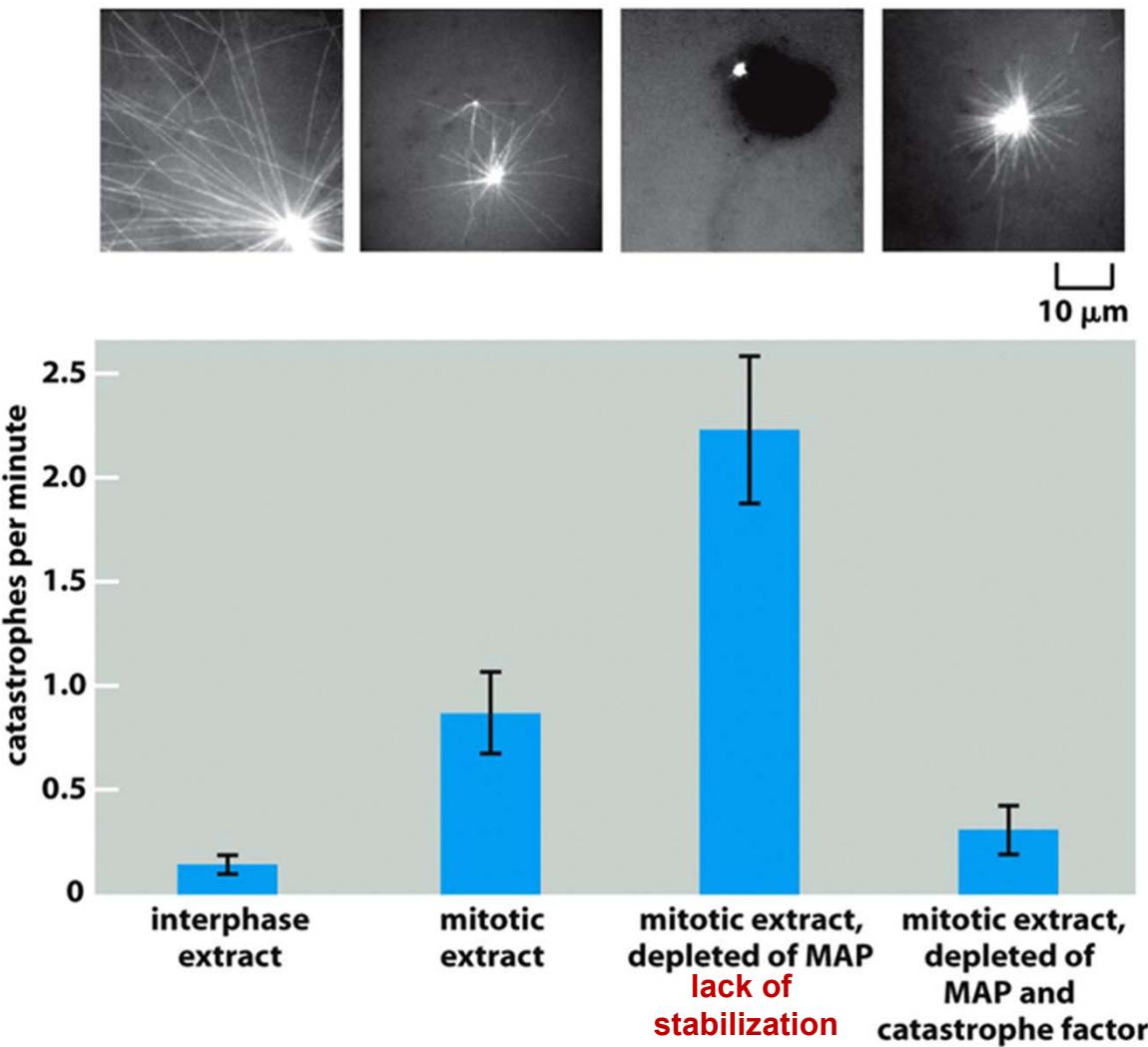
**Microtubule array** changes from “**a few long**” MTs in interphase into an array of “**many short**” MTs

Two mechanisms regulate microtubules:

- **Catastrophe factors destabilize** microtubule arrays
- **MAPs (Microtubule-associated proteins)** stabilize arrays
  - most of these factors are also regulated by phosphorylation (**M-Cdk and other mitotic protein kinases...**)

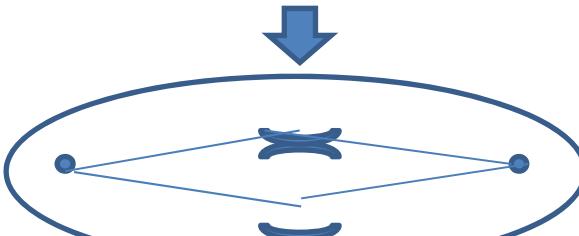
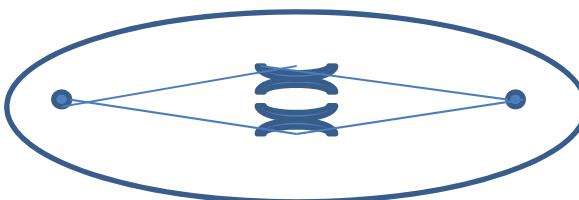


The balance between catastrophe factors and MAPs influences the length and the number of microtubules

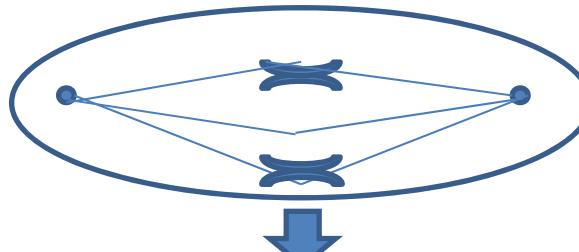


## Mitotic chromosome-promoted bipolar spindle assembly

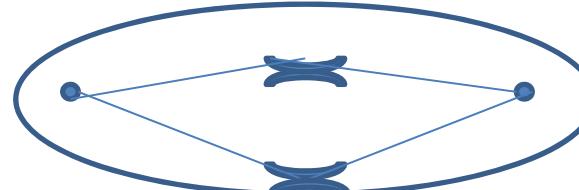
Evidence from Experiments:



**Move** one chromosome away from its position



**New** microtubules around the **new** position form



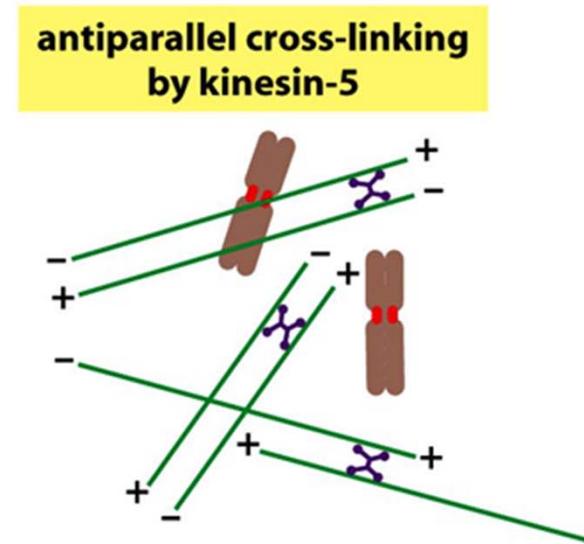
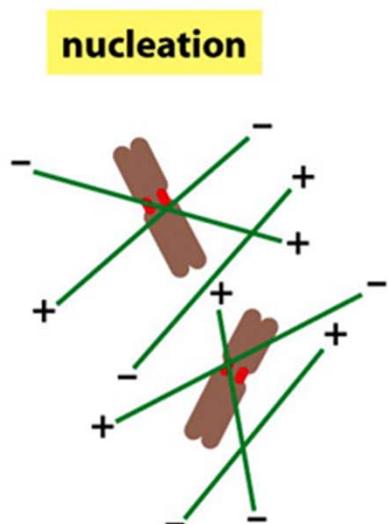
Old microtubules depolymerize

## B) Acentrosomal spindle assembly by motor proteins and chromosomes

### 1. Chromosome-bound GEF activates Ran-GTP:

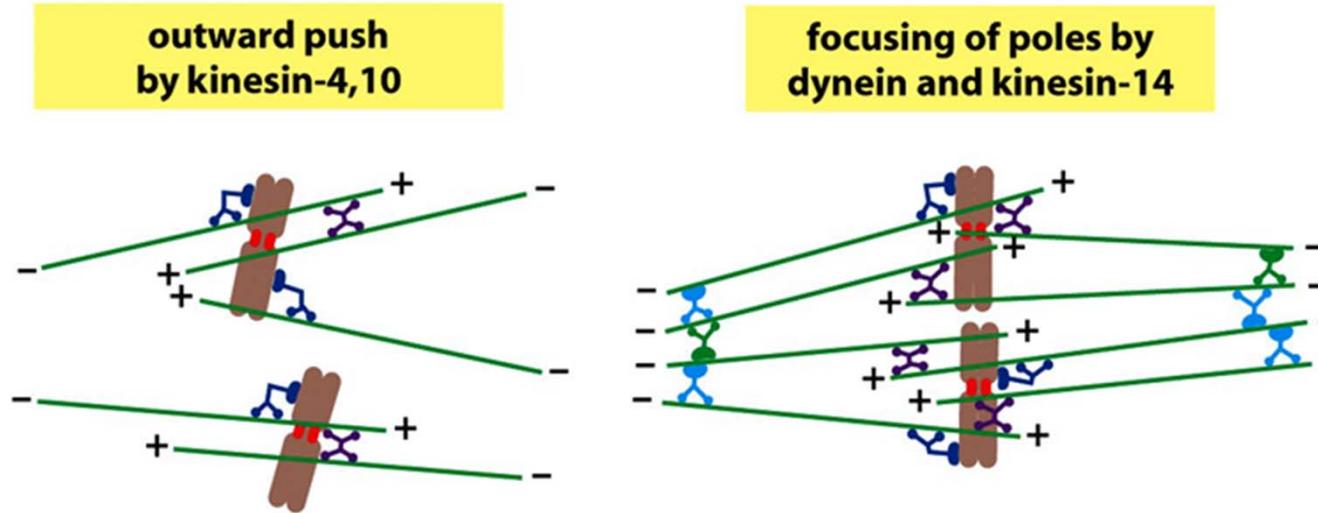
- Ran-GTP releases microtubule-stabilizing proteins
- Ran-GTP stimulates local **nucleation** and stabilizes **microtubules** around chromosomes

### 2. Kinesin-5 crosslinks microtubules and pushes minus ends towards the spindle poles (in opposite directions)



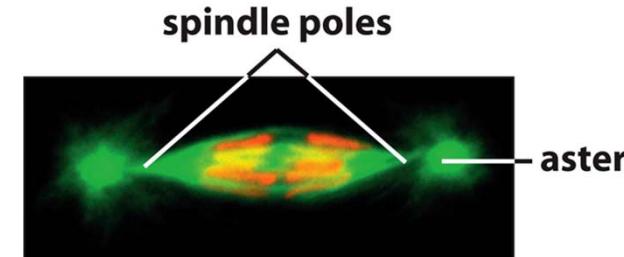
## B) Acentrosomal spindle assembly by motor proteins and chromosomes

3. Kinesins -4 and -10 push the **minus ends** away from the chromosomes
4. Dynein, Kinesin-14, cross-link and focus **minus ends** to form two spindle poles.

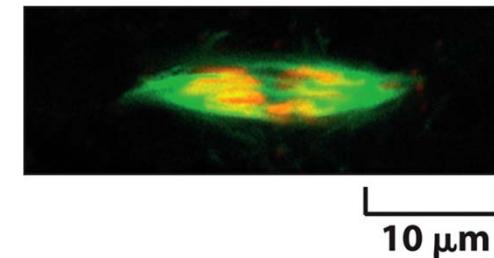


## Acentrosomal spindle assembly (Bipolar spindle assembly)

Normal centrosomal spindle



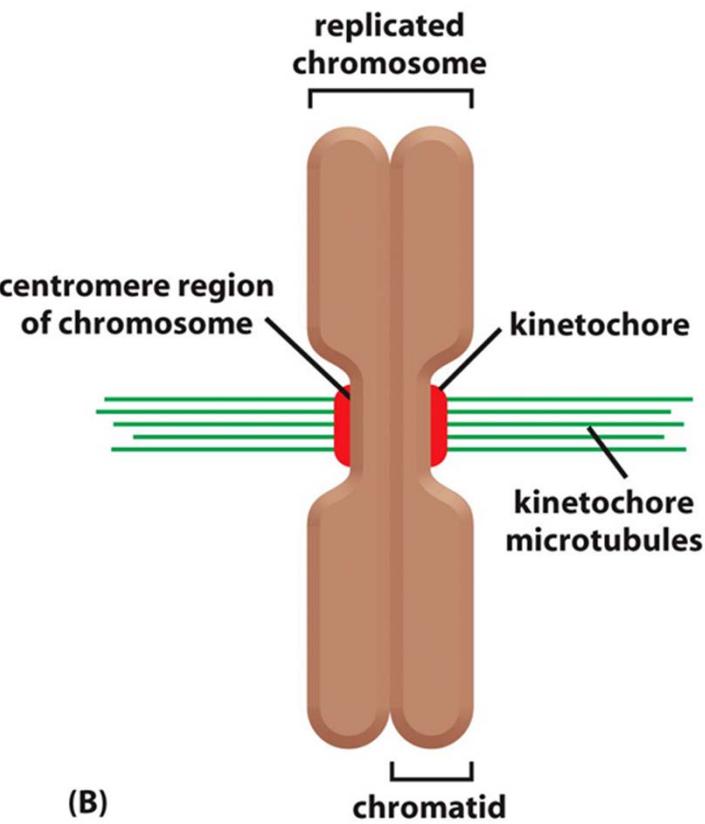
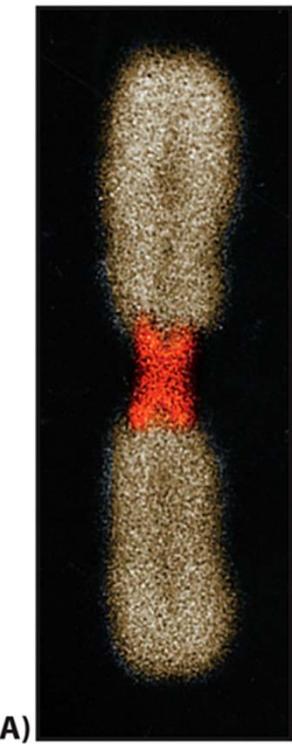
Self-organized  
acentrosomal spindle



10  $\mu\text{m}$

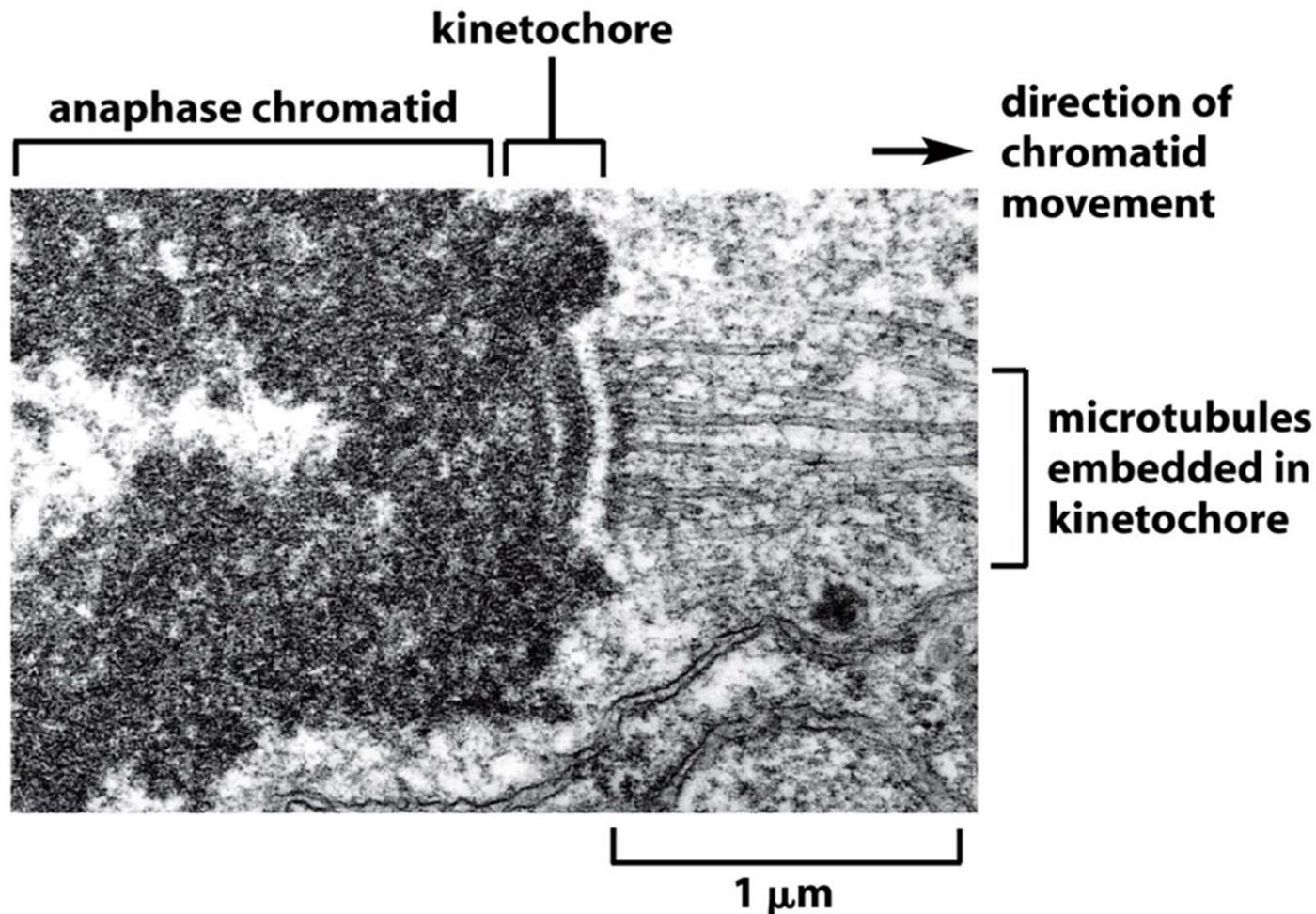
- Exists in higher plants
- Exists in many animal oocytes
- Certain insect embryos without fertilization  
(remember: centrosomes are provided by the sperm cell)
- However in animal cells, acentrosomal spindle is often mis-positioned and results in abnormalities in cytokinesis

# The kinetochores attach sister chromatids to the spindle



Immunofluorescence microscopy using antibodies against kinetochore proteins

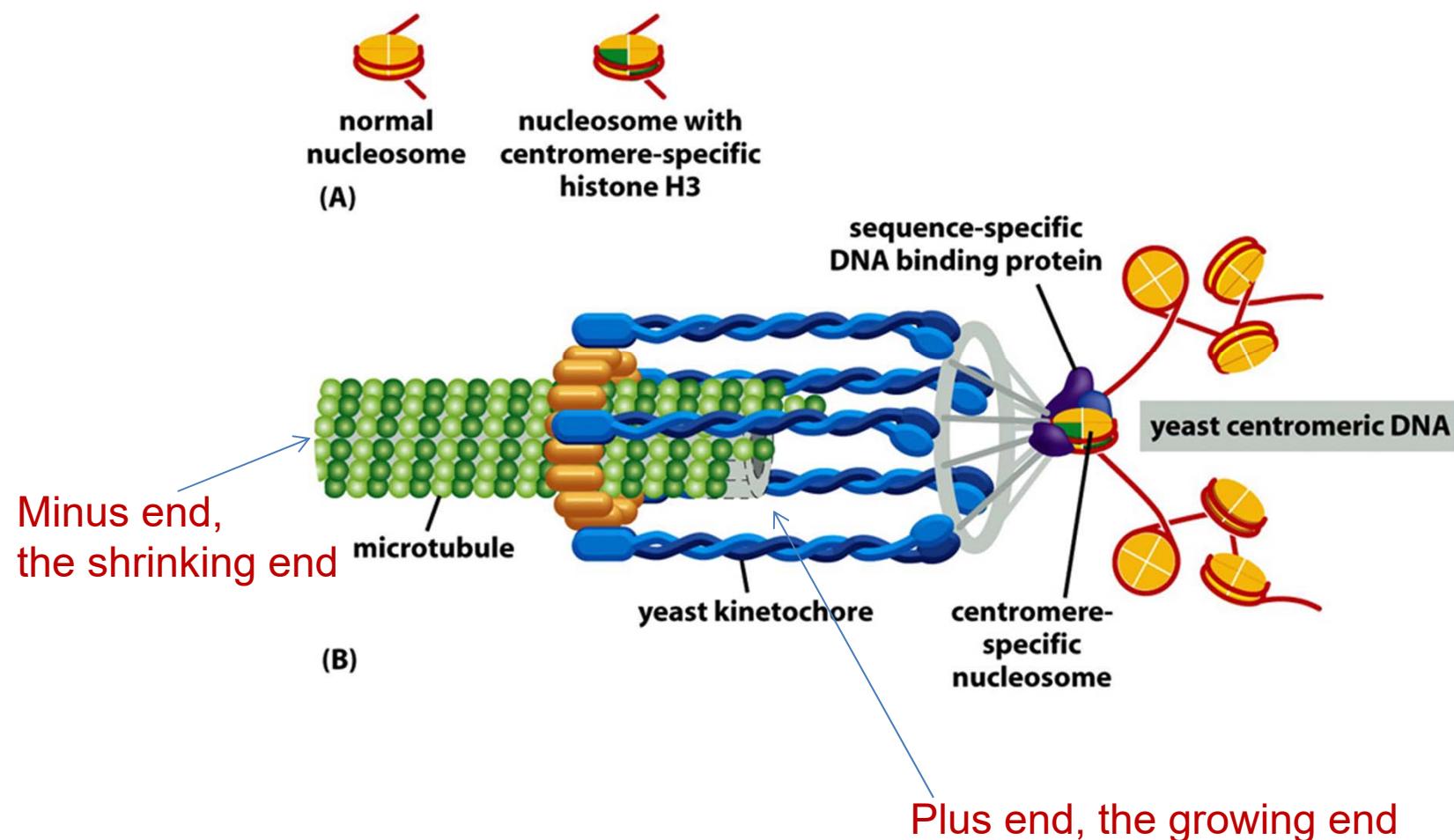
Kinetochores attach sister chromatids to the spindle



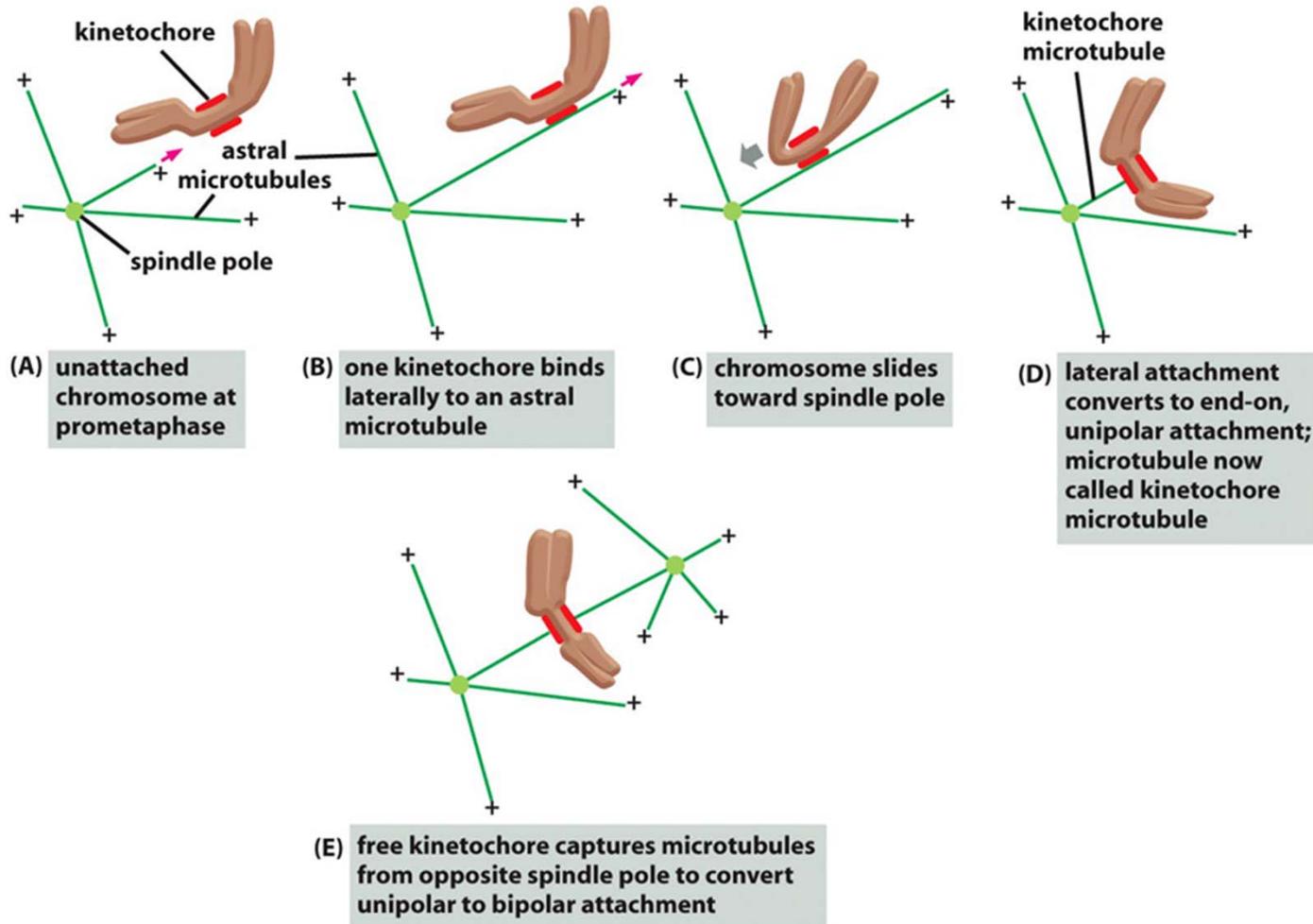
Ultrastructural analysis (electron micrograph)

# The kinetochore is the microtubule attachment site

Kinetochore: a multilayer protein structure that is built on the heterochromatin in the centromere



# The capture of centrosome microtubules by kinetochores (trial & error)



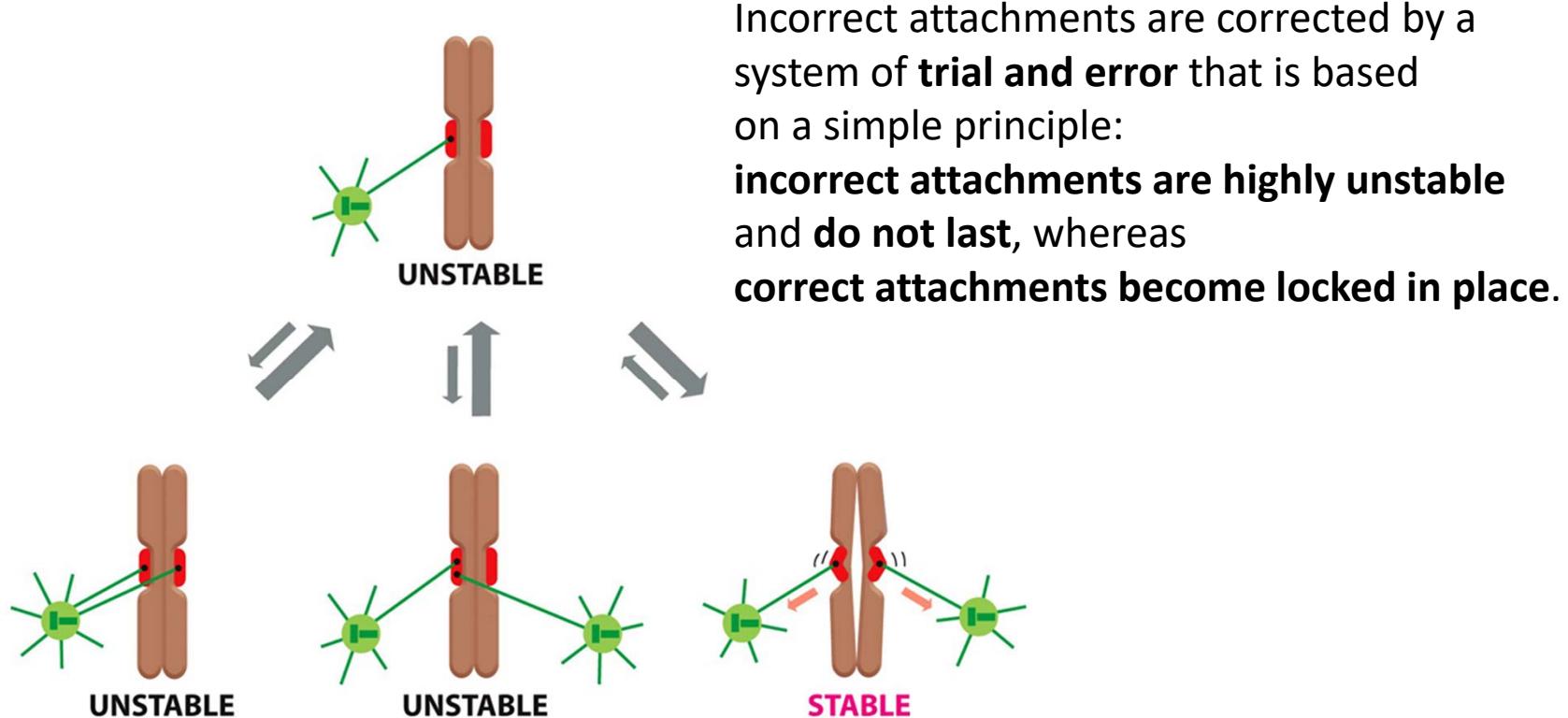
# What about acentrosomal kinetochore attachment to the microtubules?

## Three steps to success:

- **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

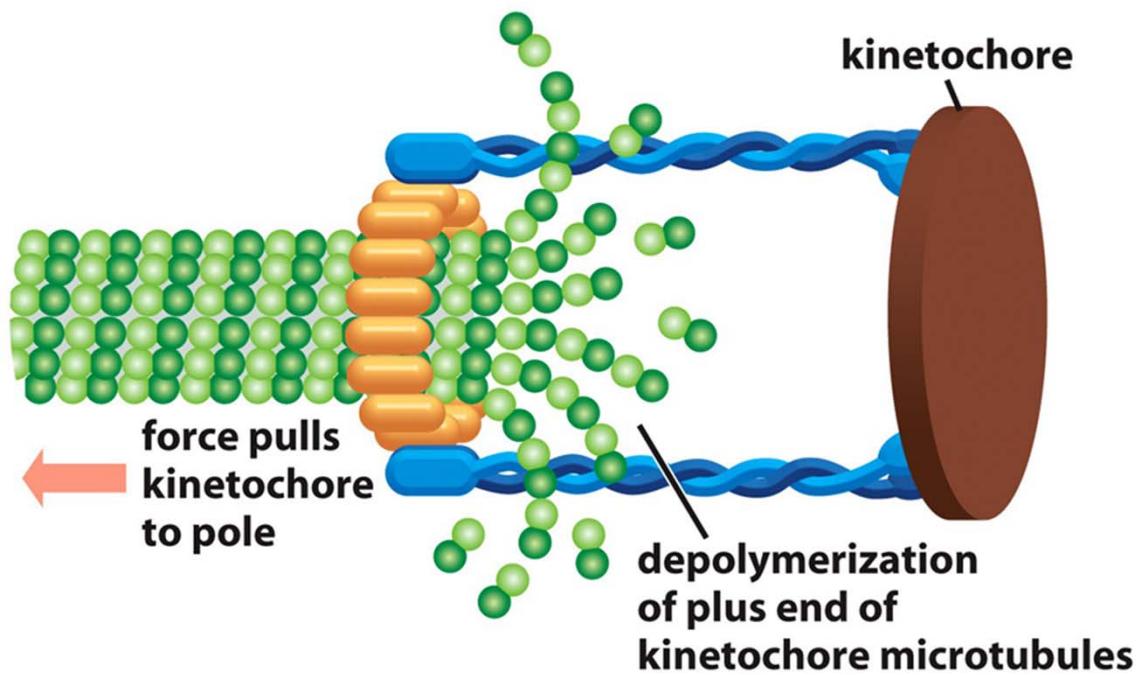


Incorrect attachments are corrected by a system of **trial and error** that is based on a simple principle:  
**incorrect attachments are highly unstable and do not last**, whereas  
**correct attachments become locked in place.**

This **tension sensing mechanism** is the checkpoint for the successful **metaphase to anaphase transition!!!**

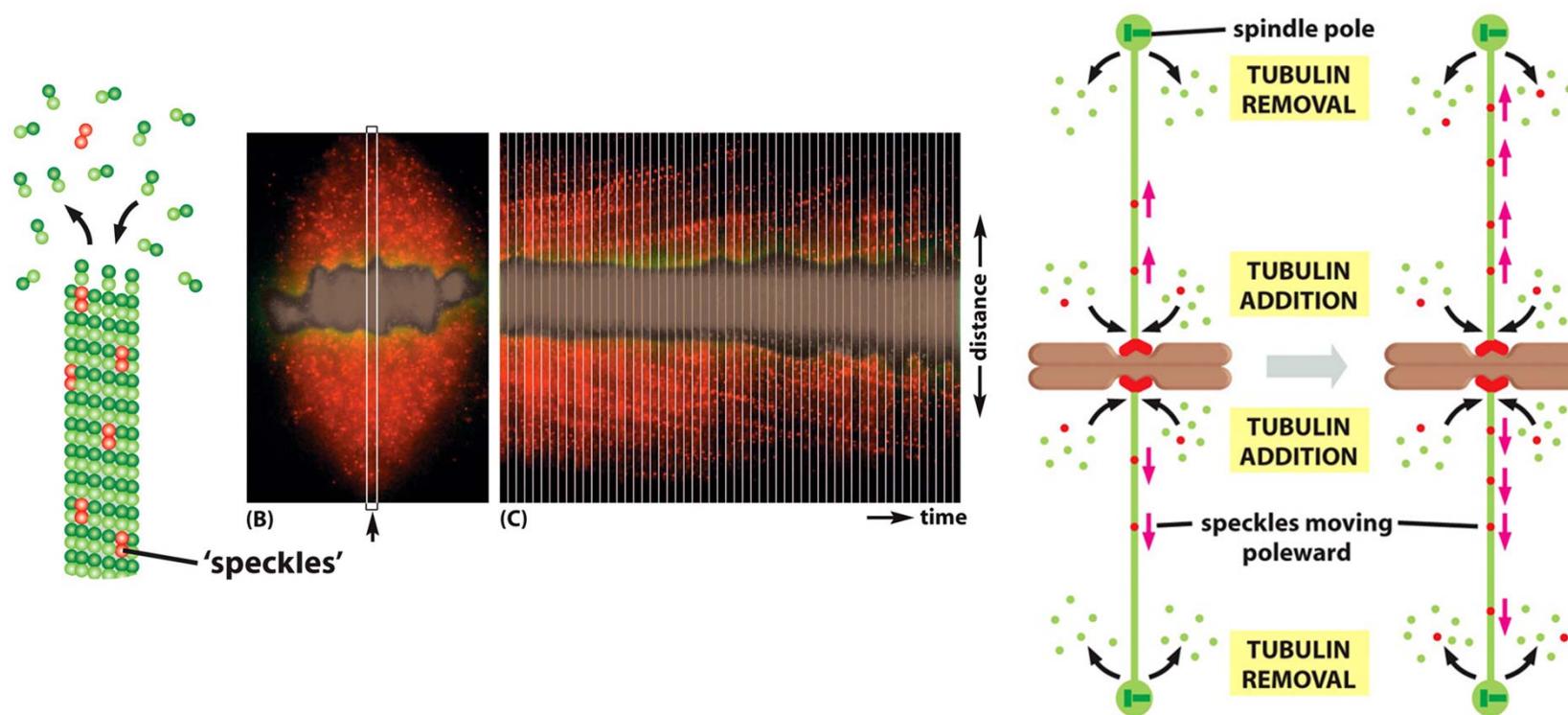
## Three major forces move chromosomes on the spindles

1. Depolymerization at the plus end of the microtubule pulls kinetochores.



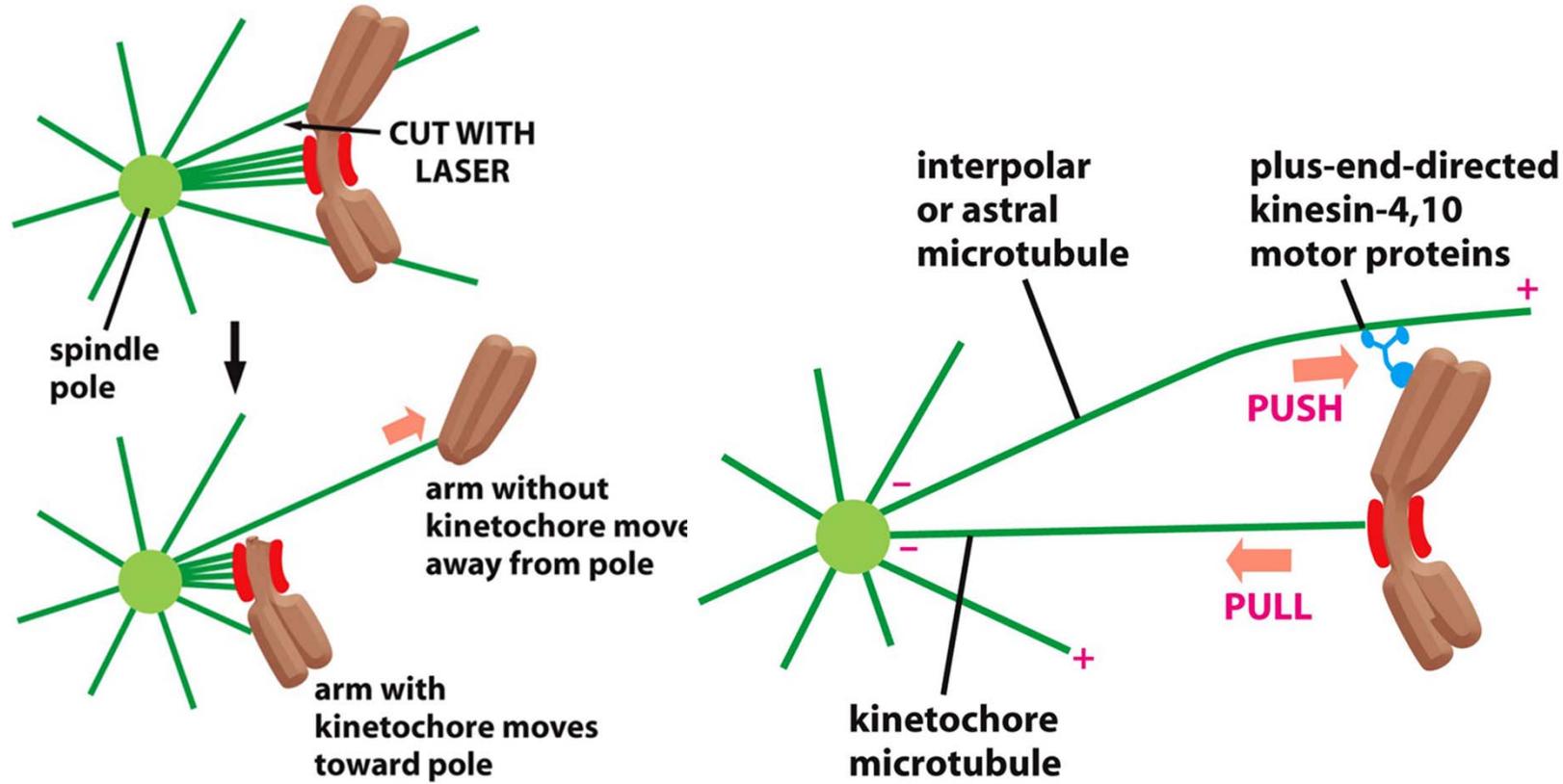
## Three major forces move chromosomes on the spindles

### 2. Microtubule flux in the metaphase spindle (depolymerization at the minus end & polymerization at plus end)



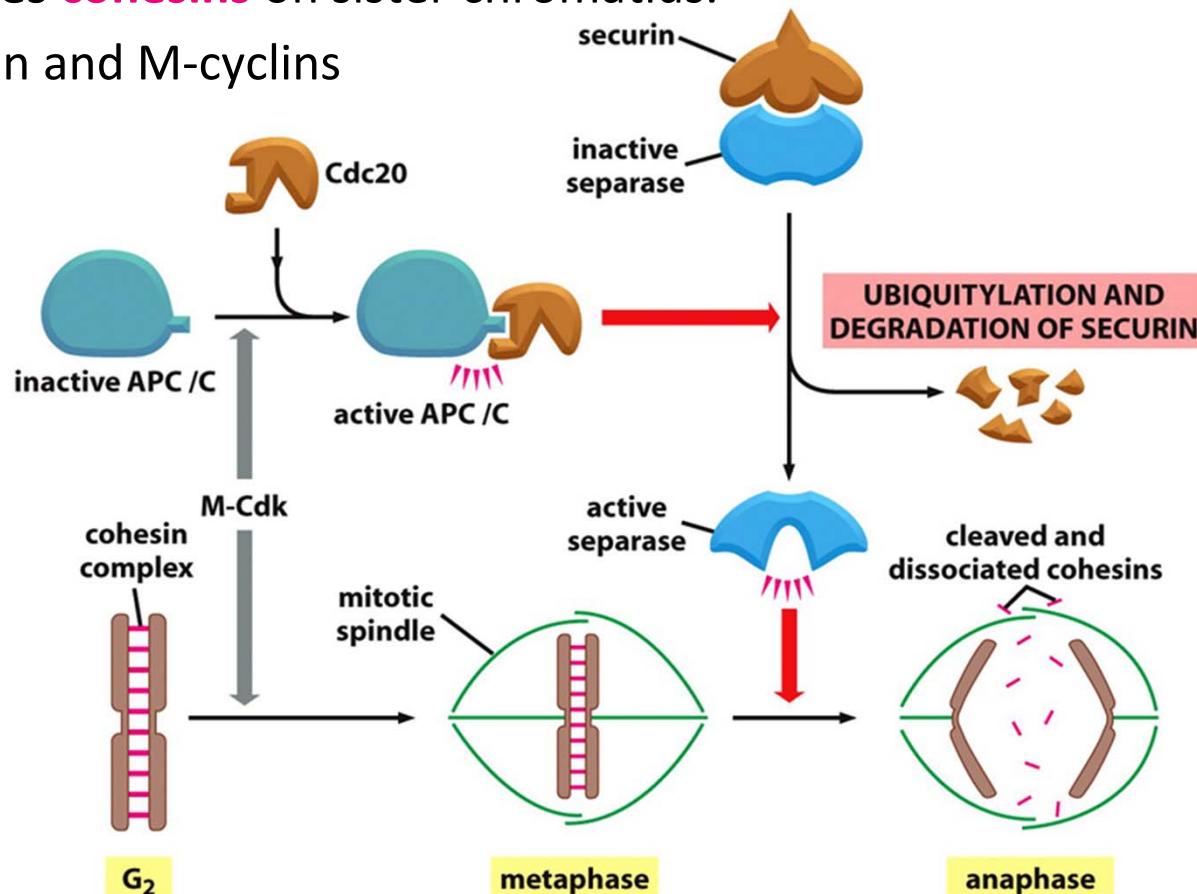
## Three major forces move chromosomes on the spindles

### 3. Polar ejection force (driven by Kinesin-4, 10) (alignment of chromosomes in prometa-/metaphase)



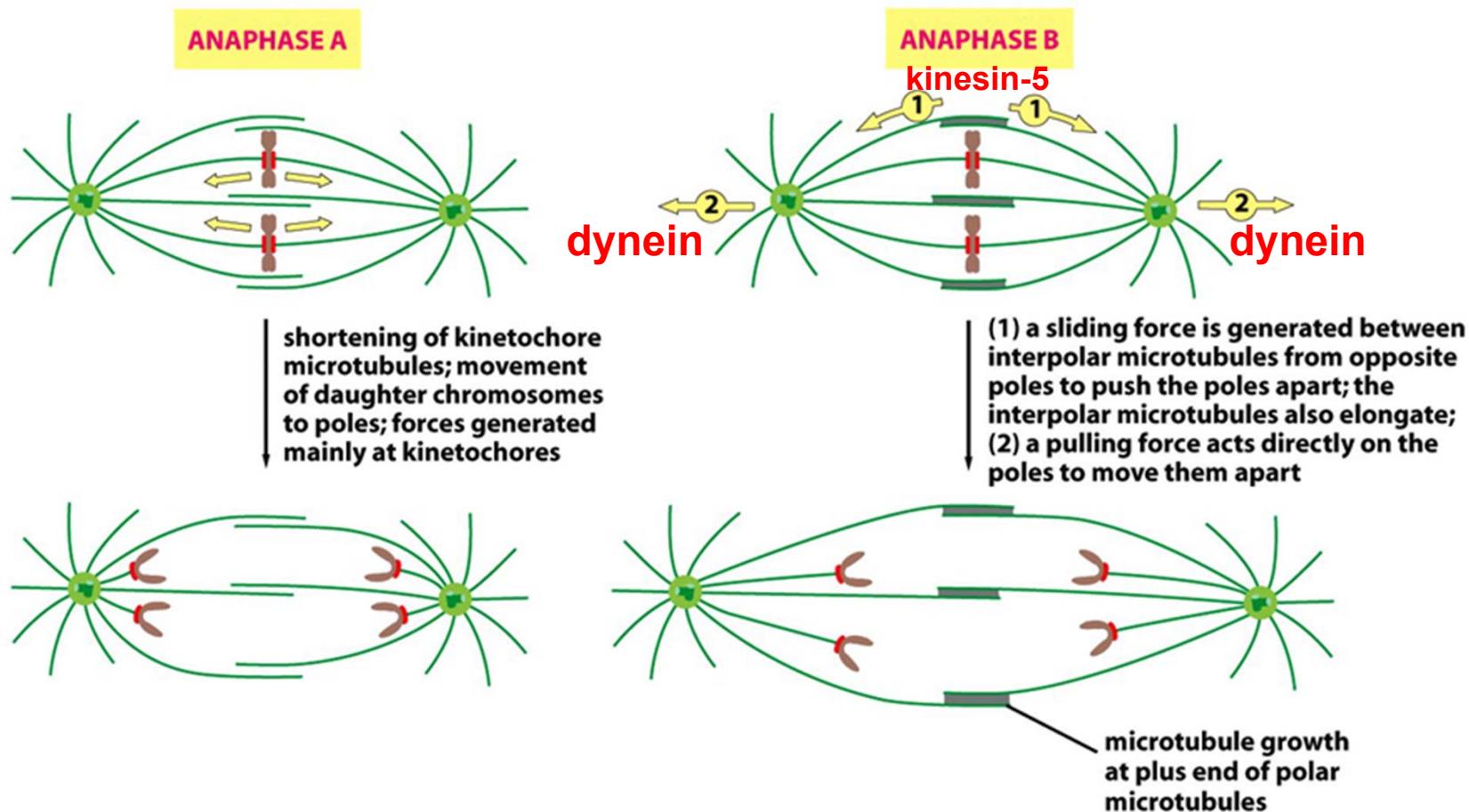
## The APC/C complex triggers sister-chromatid separation and thus the completion of mitosis

- APC/C activates the **separase** by poly-ubiquitin-mediated proteasomal degradation of **securin** (release of separase).  
**Separase cleaves cohesins** on sister chromatids.
- Degrade S-cyclin and M-cyclins



# Chromosomes segregate in anaphase in two stages

- **Anaphase A:** initial stage, shortening of the kinetochore microtubules
- **Anaphase B:** later stage, separation of the spindle poles  
**(motor proteins: kinesin-5 & dynein).**



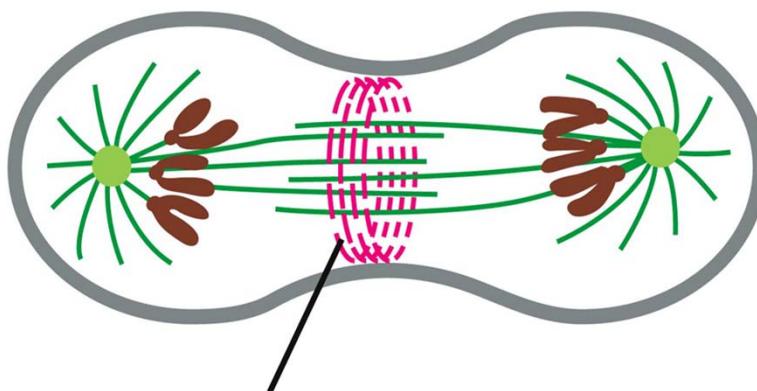
## Finally: Telophase

- **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.

## The M-phase: II. Cytokinesis (division of cytoplasm)

Cytokinesis (short version...)

**Four stages:** **initiation:** contractile ring formation  
**contraction:** myosin II/actin contraction  
**membrane insertion:** plasma membrane addition  
**completion**

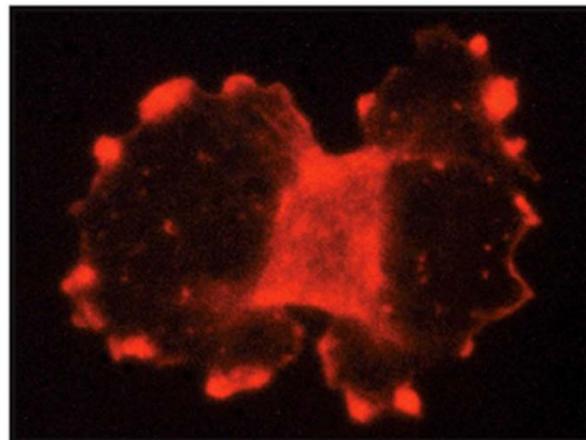


**actin and myosin filaments of the  
contractile ring**

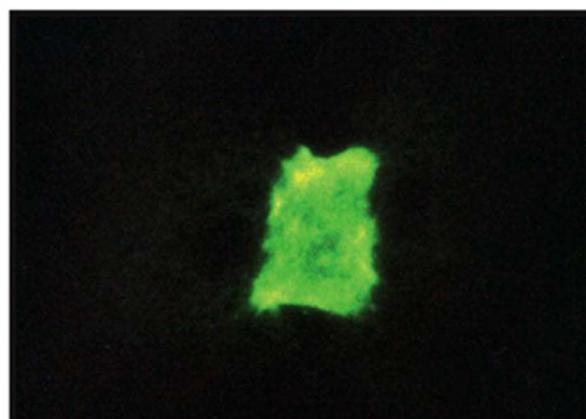


SEM: cleaving frog egg

Actin and myosin II generate the force for cytokinesis:  
- the contractile ring -



Actin in dividing cell

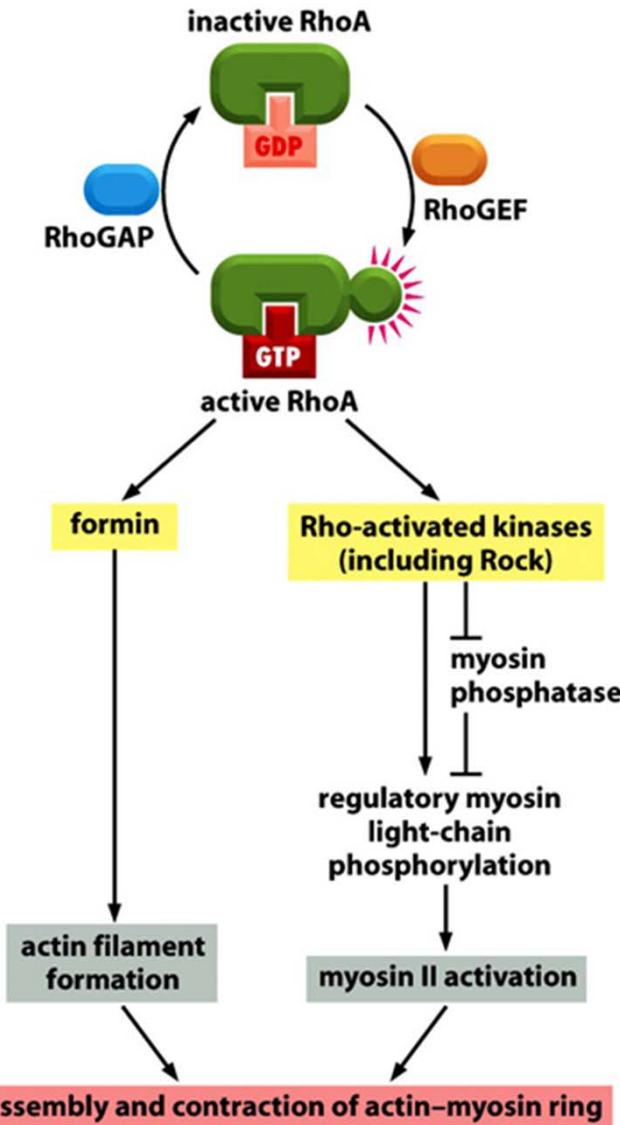


Myosin II in the same dividing cell

10  $\mu\text{m}$

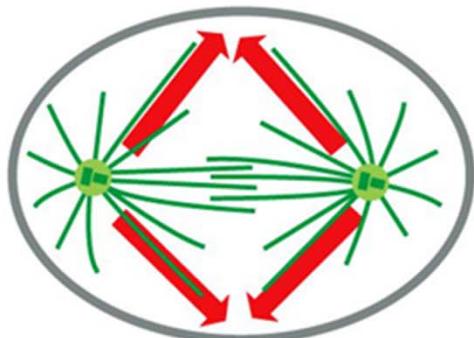
## Formation of the contractile ring: RhoA

- Actin filament formation and myosin II assembly and ring contraction is promoted by the Ras GTPase **RhoA**
- **RhoA** is activated by a **GEF** at the cell cortex
- **RhoA-GTP** activates **formin** which assembles actin filaments,
- **RhoA-GTP** activates **Rho-activated kinase (Rock)**
- Rock activates the regulatory myosin light chains (RMLCs), triggering contraction. (rehearse muscle contraction)

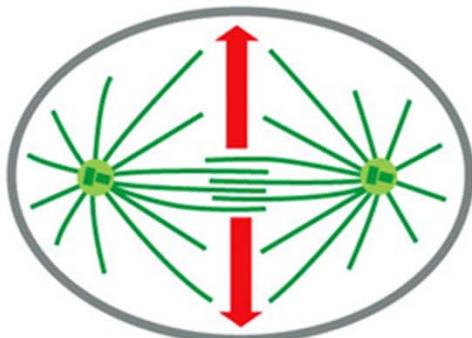


## Spindles determine the position of the cleavage furrow

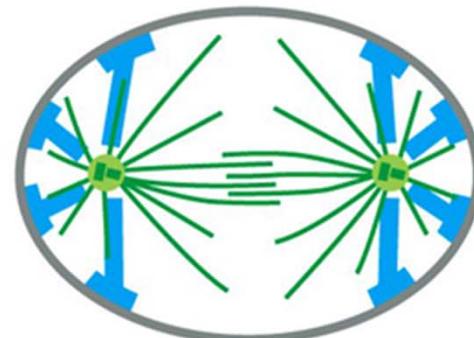
Three models have been proposed, but no agreement yet...



(A) **astral stimulation model**



(B) **central spindle stimulation model**



(C) **astral relaxation model**

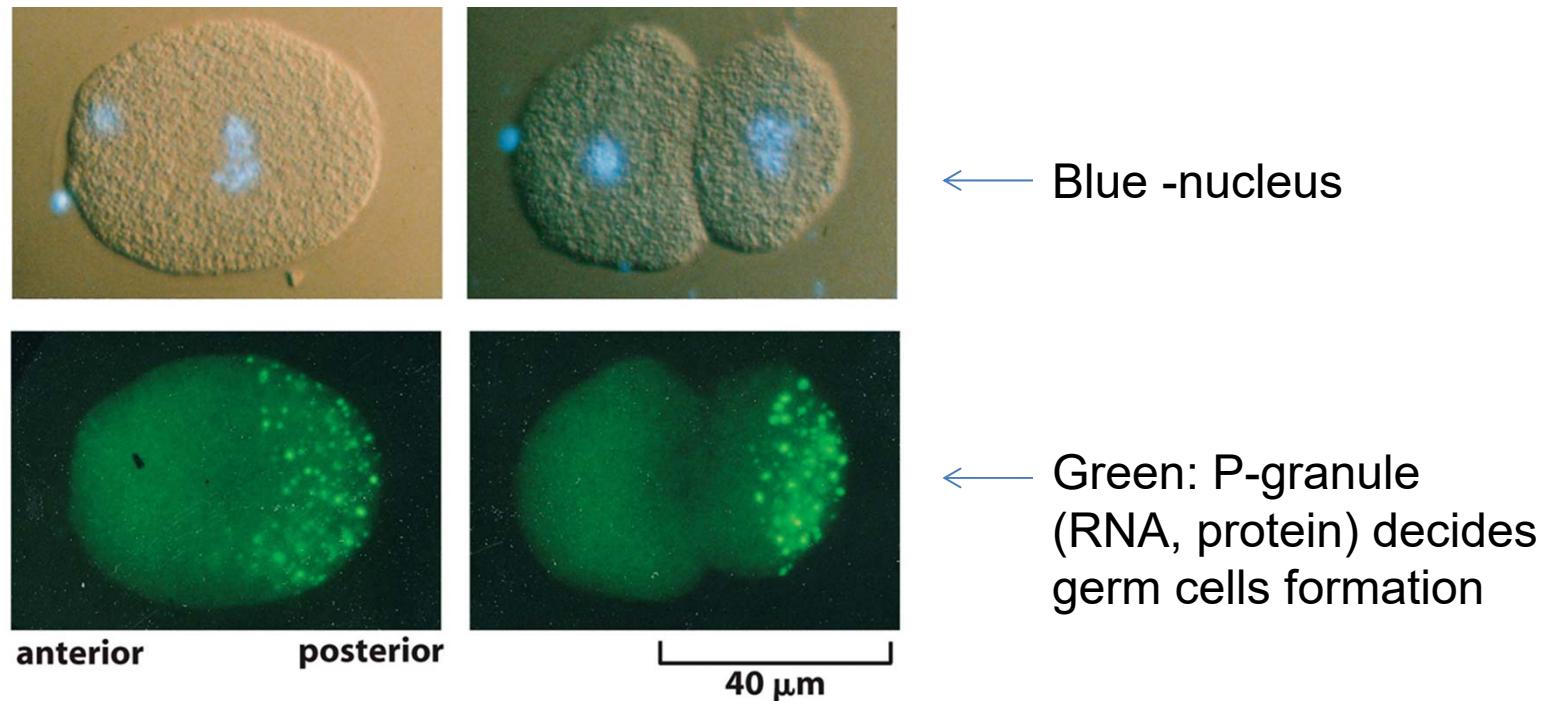
## Organelle division / distribution

- **Organelle grow from pre-existing organelle** and double in cell cycle.
- **Mitochondria and plastids** (e.g. 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.  
The Golgi apparatus is associated with the spindle poles.

## Symmetrical and asymmetrical division

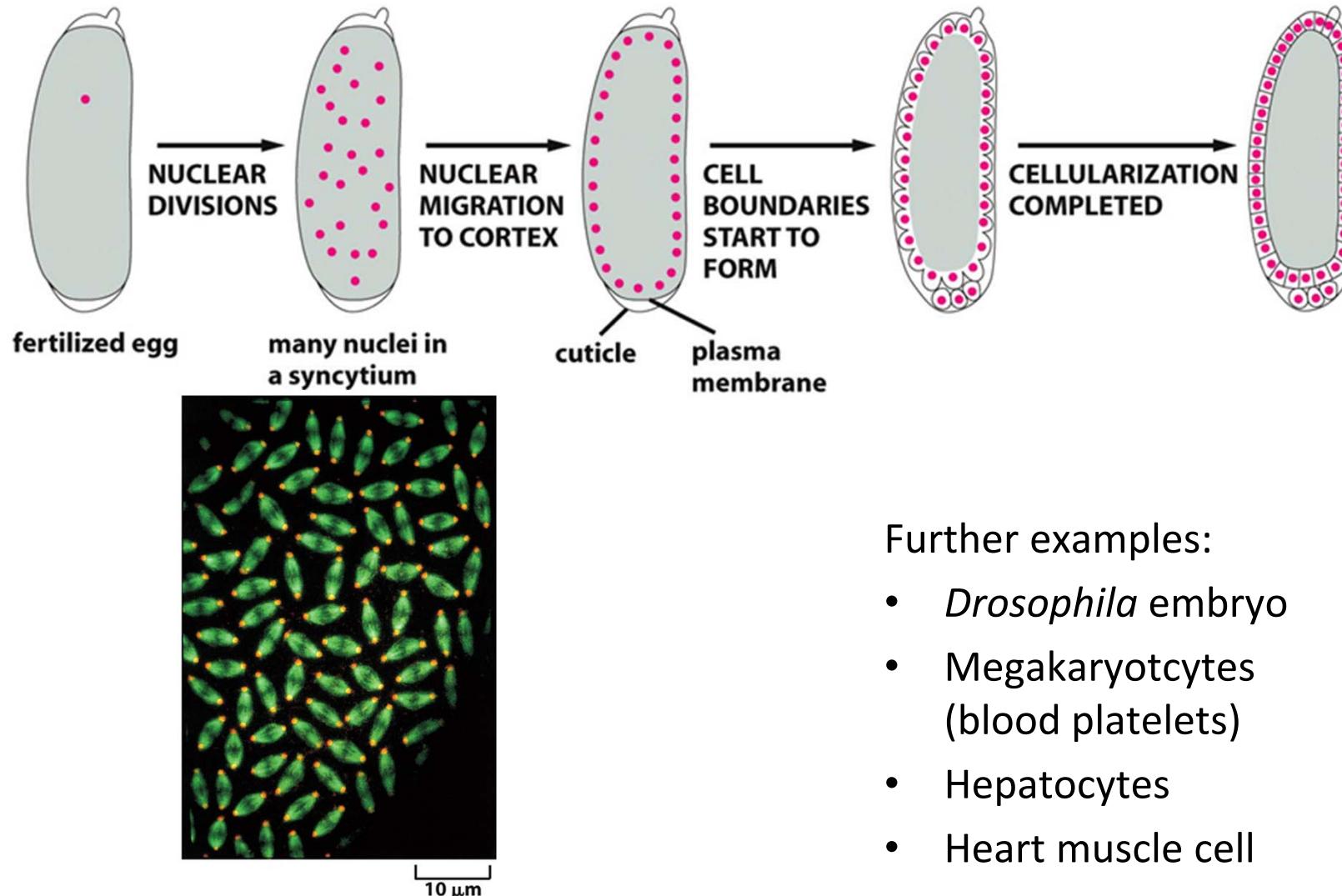
- Most divisions are symmetrical
- During development, asymmetrical division is typically associated with different fate in cell development, the spindle positioning is important and nicely controlled

### Asymmetrical division: fertilized egg, *C. elegans*



# Mitosis without cytokinesis: *Drosophila* embryo

Syncytium and cellularization for *Drosophila* embryo



Further examples:

- *Drosophila* embryo
- Megakaryocytes  
(blood platelets)
- Hepatocytes
- Heart muscle cell

### III. Control of cell division and cell growth

Important aspects and many questions:

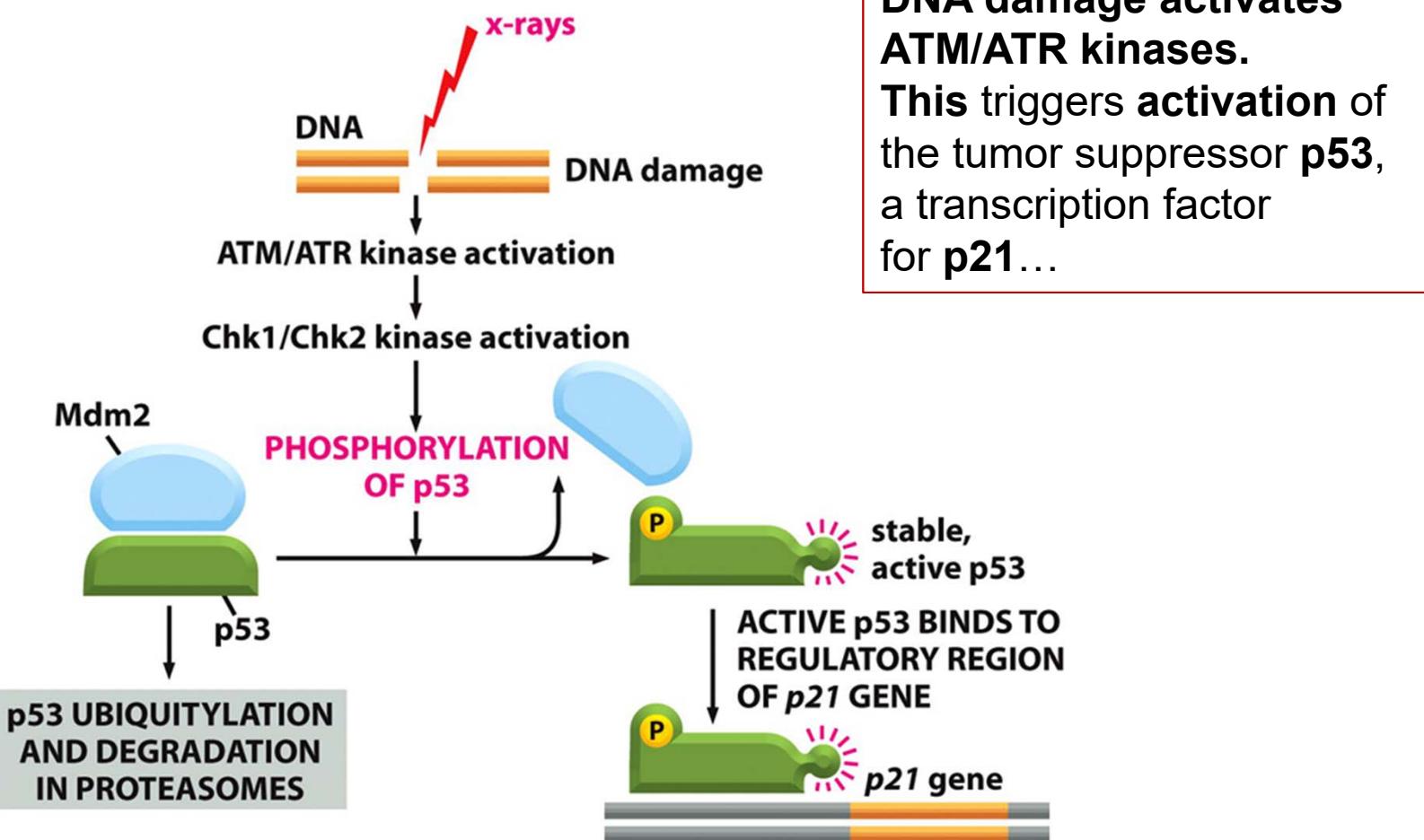
- How is cell growth and division controlled?
- How can DNA damage block cell division?
- How can mitogens stimulate cell division?
- How is cell growth and cell division coordinated?

## Control of cell growth and division

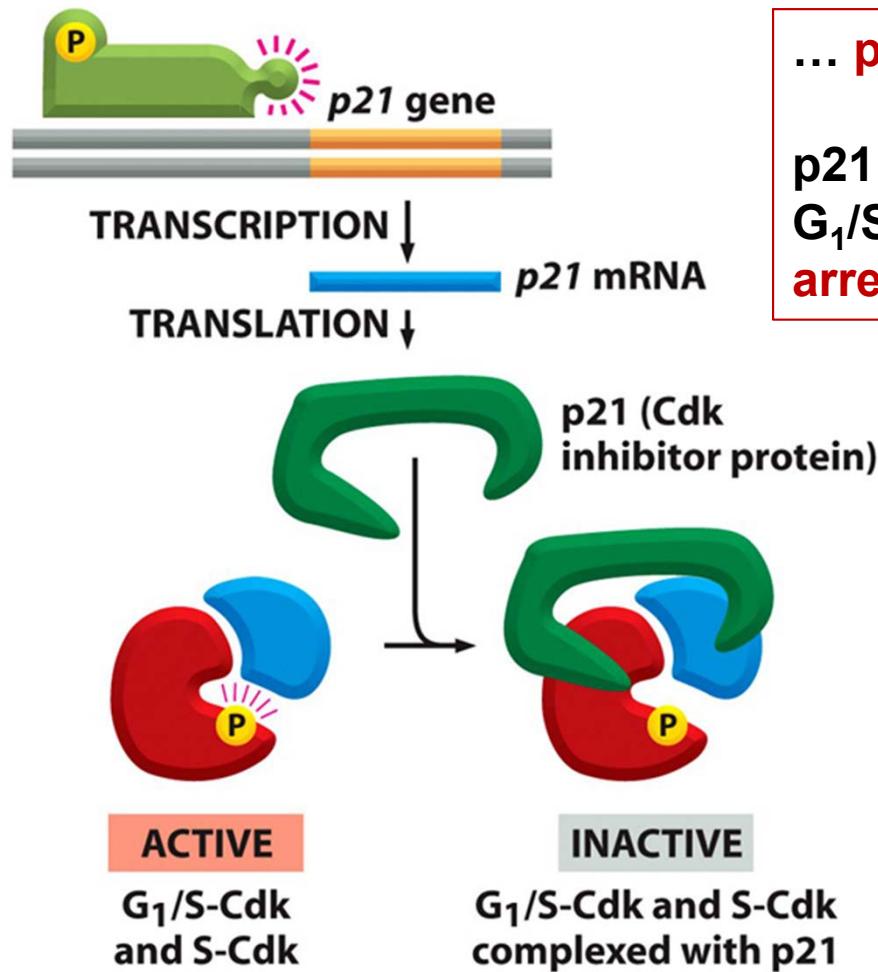
What controls organ size and organism size?

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

# DNA damage response triggers cell cycle arrest: activation of the tumor suppressor p53



# p21 synthesis triggers the arrest of the cell cycle: both at G<sub>1</sub>/S and G<sub>2</sub>/M transition.



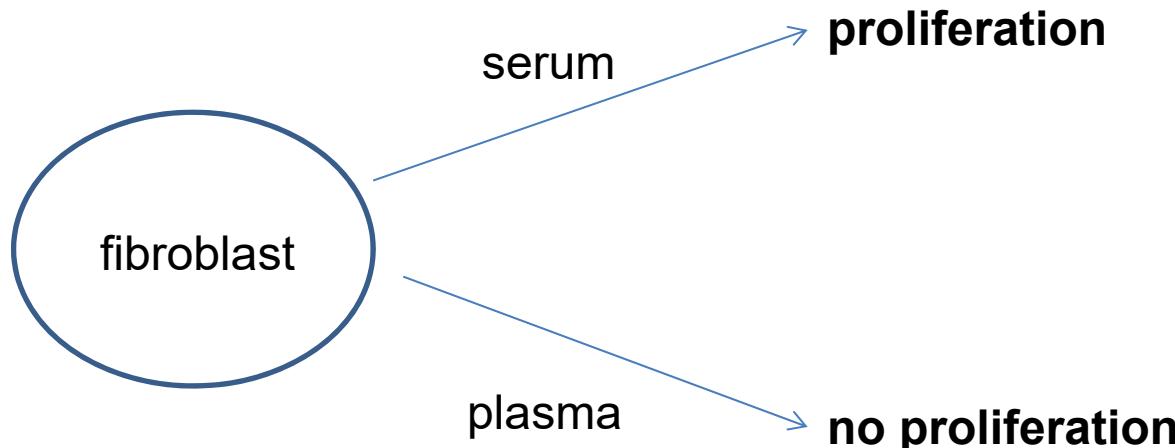
... **p21 is a Cdk inhibitor protein!**

**p21 triggers inactivation of G<sub>1</sub>/S-Cdk and S-Cdk and thus arrests the cell cycle**

## Defects in DNA damage response have severe consequences

- **ATM mutation:** Ataxia-telangiectasia (**Louis–Bar syndrome**)
  - 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

Cell division of multicellular organisms needs stimulation by mitogens



What is the difference between serum and plasma?

Both are derived from blood without cells, but:

**serum** is the supernatant **after blood clotting**,

**plasma** is the supernatant **prior to blood clotting**.



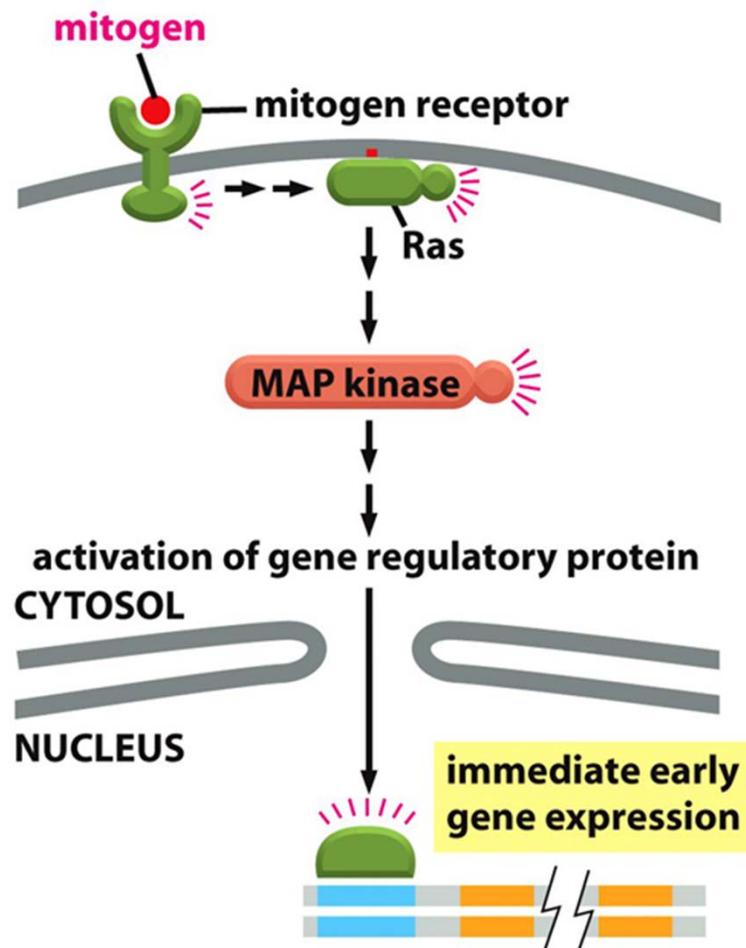
Platelet during blood clotting release the **mitogen PDGF**  
(platelet-derived growth factor)

## Facts about mitogens:

- Over 50 different mitogens have been identified yet: 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

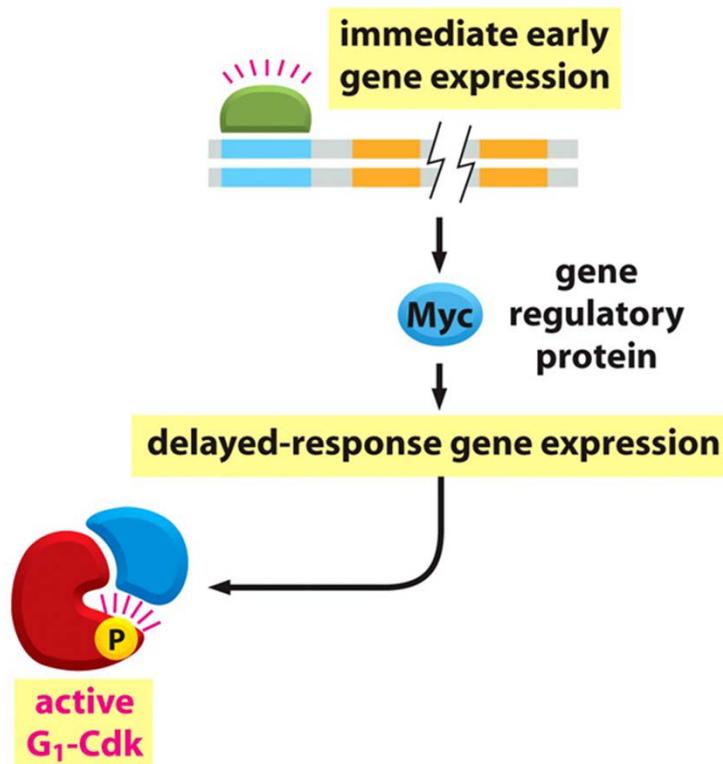
Mitogens control the rate of cell division in the G1 phase of the cell cycle



## Signaling pathway:

- **mitogen** activates receptor
- receptor activates **Ras**
- **Ras** activates the mitogen-activated protein (MAP) kinase cascade
- The **MAP kinase cascade** activates transcription factors and triggers immediate early gene expression
- one of these genes is the gene regulatory protein (transcription factor) **Myc**...

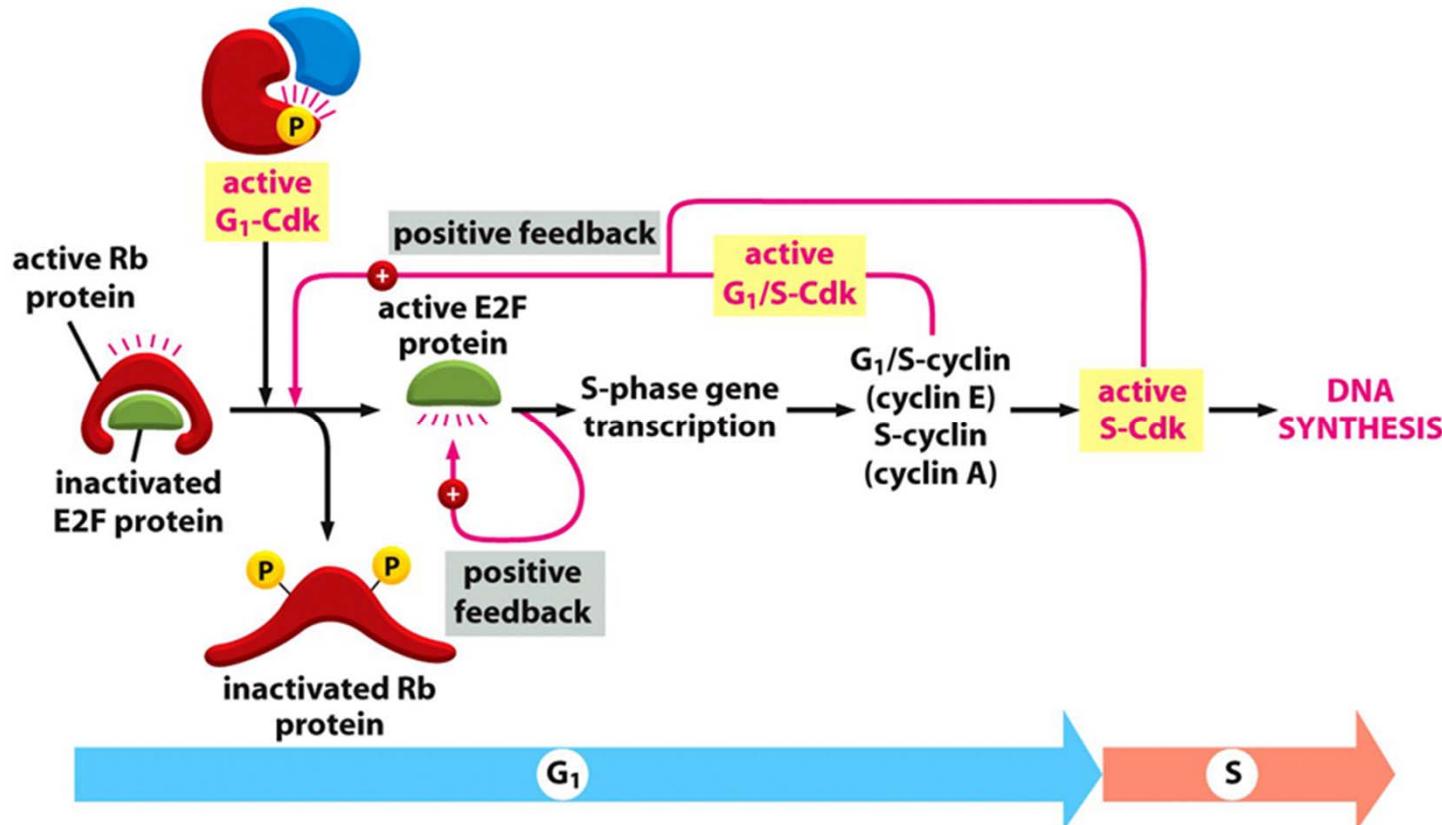
# MAP transcriptionally activates c-Myc



## Signaling pathway:

- the gene regulatory protein (transcription factor) **Myc** promotes expression of **G<sub>1</sub> cyclins (D cyclins)**
- **G<sub>1</sub> cyclins (D cyclins)** activate **G<sub>1</sub>-Cdk** and trigger cell cycle entry
- but **G<sub>1</sub>-Cdk** activates also other transcription factors e.g. the **E2F proteins.....**

# Rb-E2F pathway in cell cycle control



## Signaling pathway:

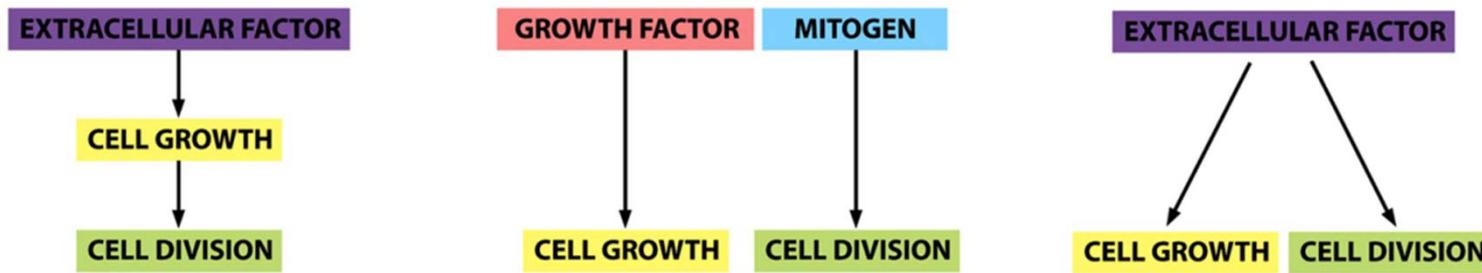
**G<sub>1</sub>-Cdk** activates **E2F proteins**, which activate transcription of S-phase proteins like **G<sub>1</sub>/S-cyclin & S-cyclin**, resulting in further activation of **G<sub>1</sub>-Cdk & S-Cdk** and thus further inactivation (phosphorylation) of the Rb protein (positive feedback loop) and the cell cycle starts....

## 4. Coordination of cell growth and cell division

Growth and division is not always coupled:

- terminally differentiated cells (muscle and nerve cells) grow but do not divide
- drosophilae fertilized eggs divide without growth.

➤ Several coordination mechanisms must exist:



Extracellular factors (nutrition)  
determines **both**, growth rate  
and the rate of cell division

The rate of growth and cell division is controlled by  
**separate**  
**extracellular factors**

Extracellular factor  
detrmines  
growthrate and the rate of  
cell division via **different**  
**signaling pathways**

Many open questions remain,  
lots of work is left to be done....

- How is cell size precisely controlled?
- How is the number of cell divisions precisely controlled?
- How to maintain the cellular homeostasis in an organism?

