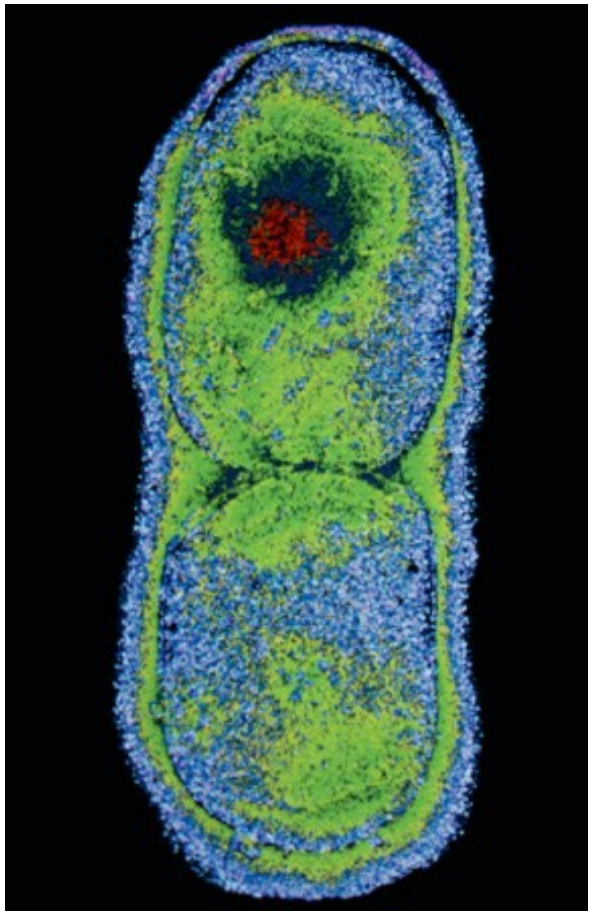
Cell cycle, cell division and its regulation

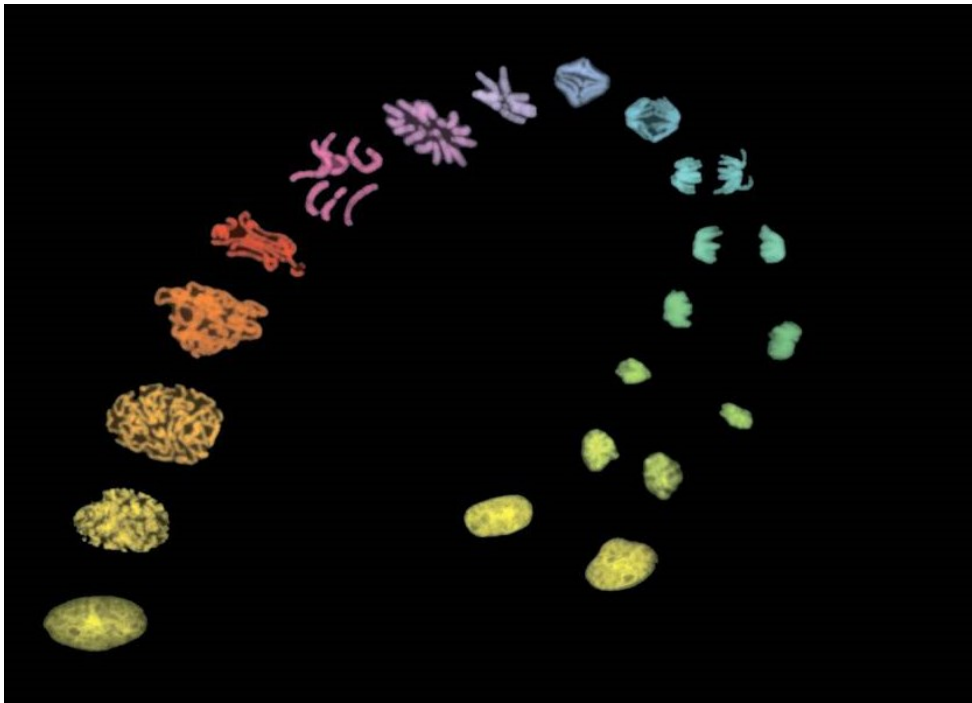


1. Cell division
2. Cell cycle
3. Mitosis, its individual phases
4. Meiosis, fundamental differences from mitosis
5. Control of cell division and cell growth

What is cell division?

- arranged sequence of macromolecular processes in which the **cell replicates its content** and then **splits into two daughter cells**, and each of them bears the same chromosomes

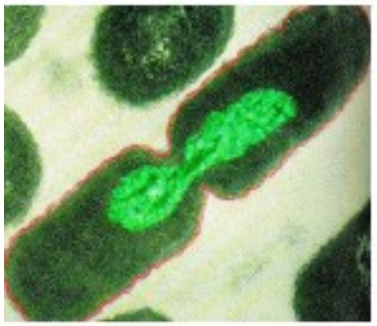
- main goal: **the reproduction of genetic material for the next generation**



Cell division in unicellular

Unicellular:

Harmonization of cell division with growth



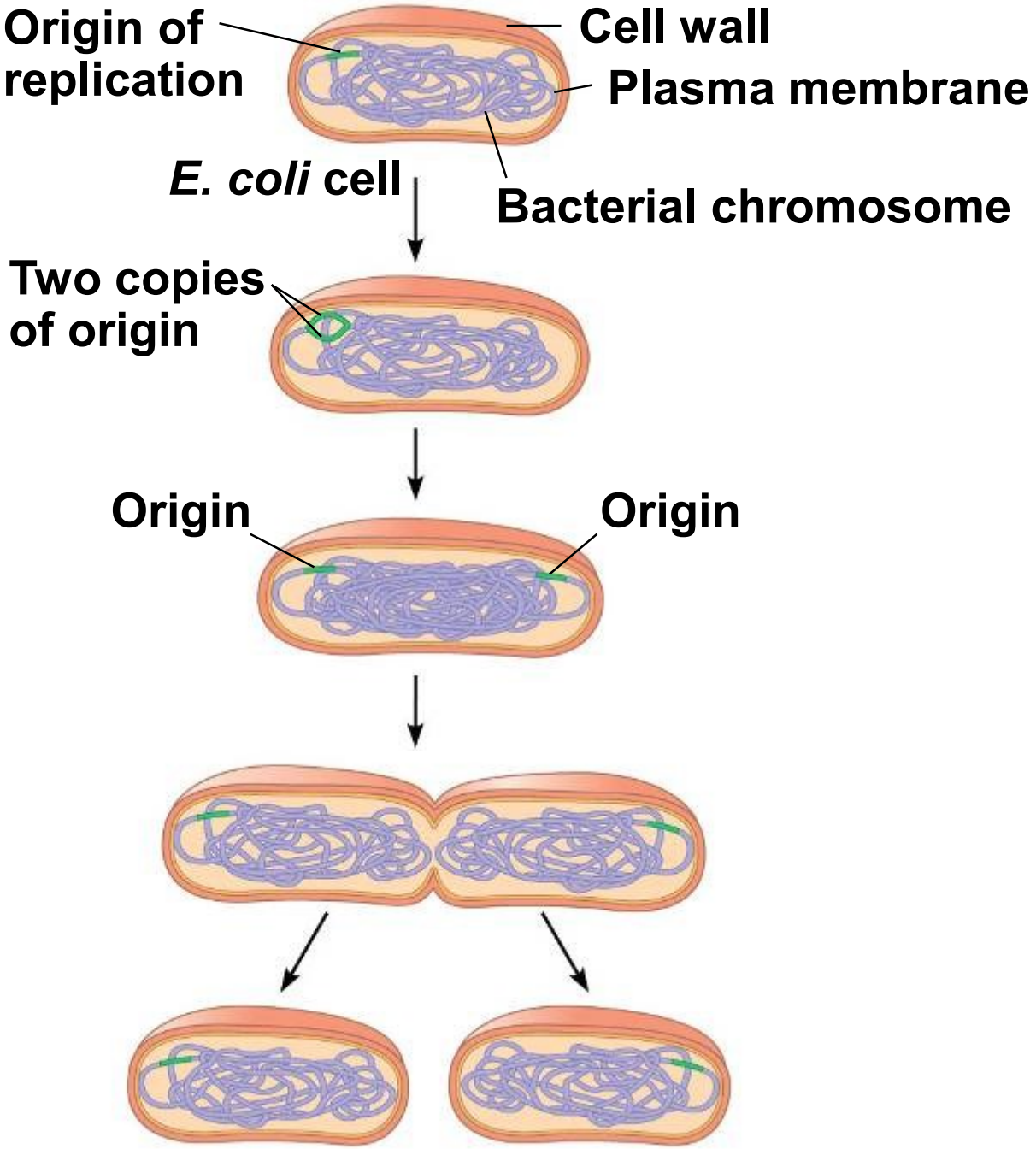
Cell division in bacteria *(E.coli)*

It has a single circular chromosome, which is attached to the plasma membrane and remains attached to it during chromosome replication.

Both chromosomes are separated from each other by cell growth. Cell wall and plasma membrane goes between between chromosomes → two cells are formed.

= **BINARY FISSION**

Figure 12.12-4

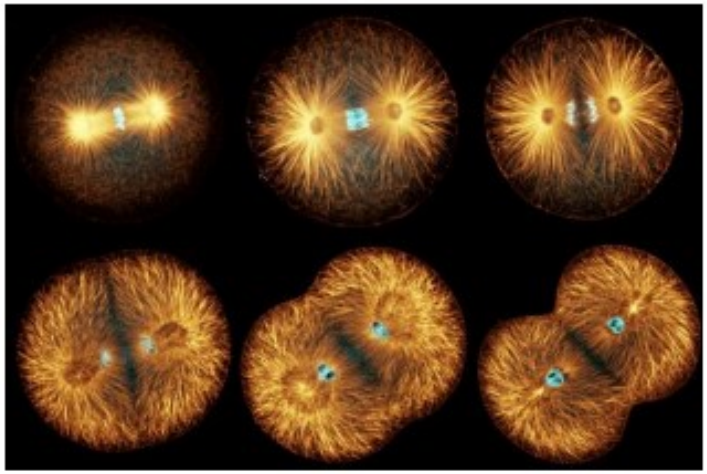


Cell division – cell cycle in multicellular

- at the beginning of the development of a multicellular organism is a single cell
- cell division necessary for
  - **growth of the organism**
  - **replacement of damaged and old cells**
  - **the spread of genetic information: mitosis, meiosis**
- before the division the cell must enlarge, chromosomes duplicated and ensure their accurate division into daughter cells
- Coordination of these processes t**akes place within the cell cycle**
- cell division is strictly controlled to maintain the unity of multicellular organism
- the loss of dividing control leads to abnormal development and may **cause tumors**

In adults the cells divide only in such places and at the time when it is needed (e.g. replacement of dying cells, restoring the injured tissue)

- **Reproduction**
- **Development and growth**
- **The renewal of tissues**

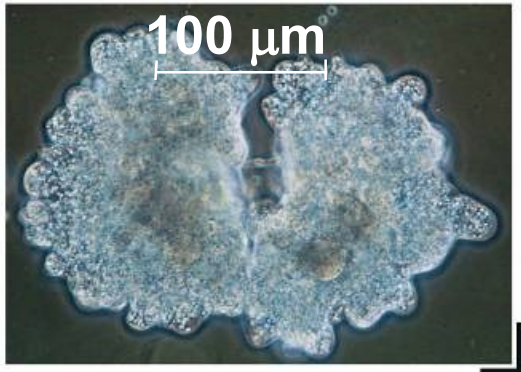


Eukaryotic cells

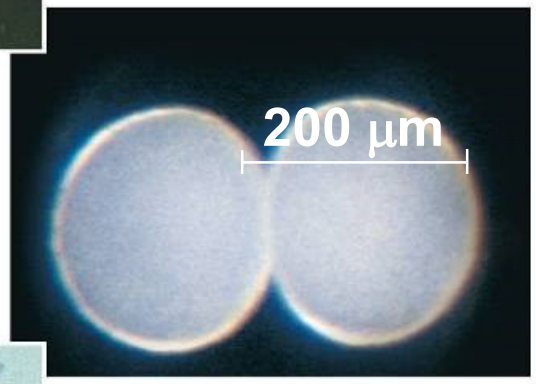
Usually divide at all:
neural, muscle

Minimum is divided:
liver cells (1 per year)

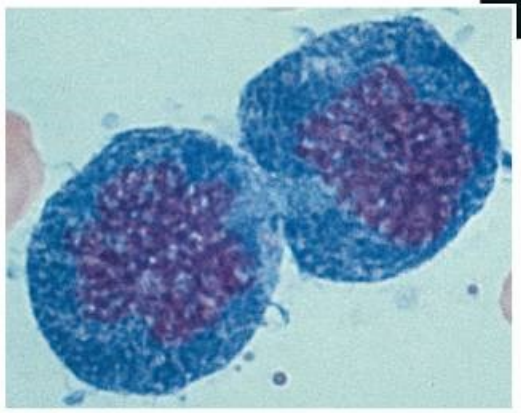
Intensively divided:
intestinal epithelial cells, blood precursor cells (more than 1 day).



◀(a) Reproduction



▶ (b) Growth and development



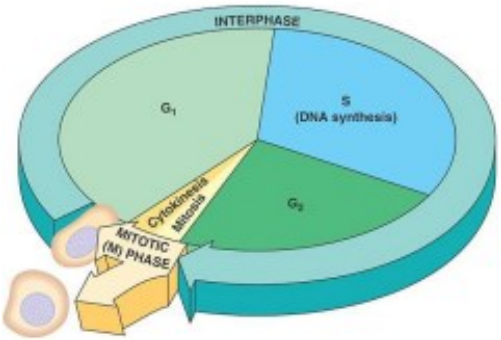
◀(c) Tissue renewal

Each of us creates million new cells every second , division arrest leads to death.

Cell cycle

cycle of cell life processes that begin with her birth and ends with the formation of daughter cells

• precise continuity of cellular processes, two distinct phases:



Interphase

• cell growth, cell organelles duplication, increased metabolism

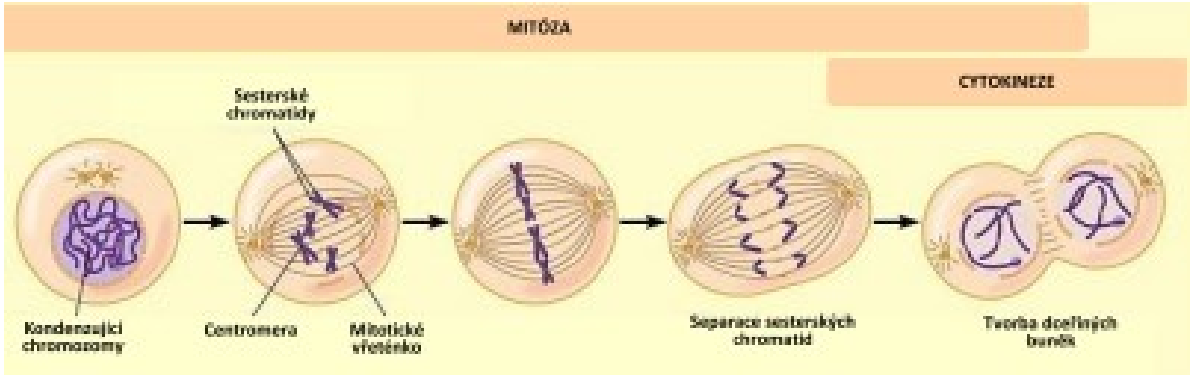
G1 first growth phase, the active synthesis of RNA and proteins
cell accumulates energy and prepares for DNA synthesis
(Replication proteins, histones)

S The synthesis of DNA, chromosomal duplication

G2 second growth phase continues synthesis of RNA and proteins
cell growth and preparation for M phase (mitotic spindle)

M phase

• mitosis (division of the nucleus)
and cytokinesis
(Cell division)
• chromatin condensation
• breakdown of the nuclear membrane
• Creation of spindle and kinetochore
• chromatid separation
• breakdown of spindles
• chromatin decondensation
• formation of membrane, cell division

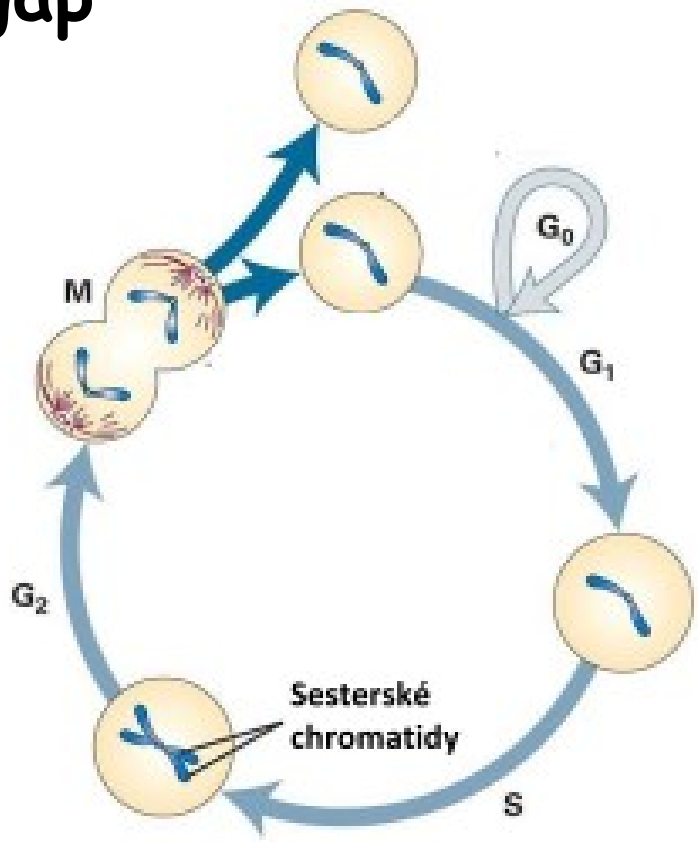


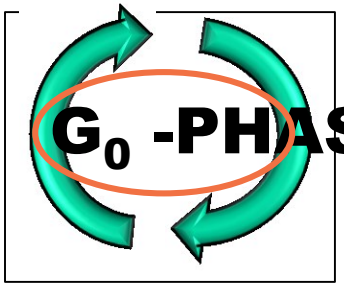
Phase of the cell cycle of eukaryotic cells

INTERPHASE (G1, S, G2) (normal cellular activity)
M-PHASE (cell division)

G = gap

G1 and G2 phases are phases when cell growth, duplication of cytoplasmic organelles are in progress

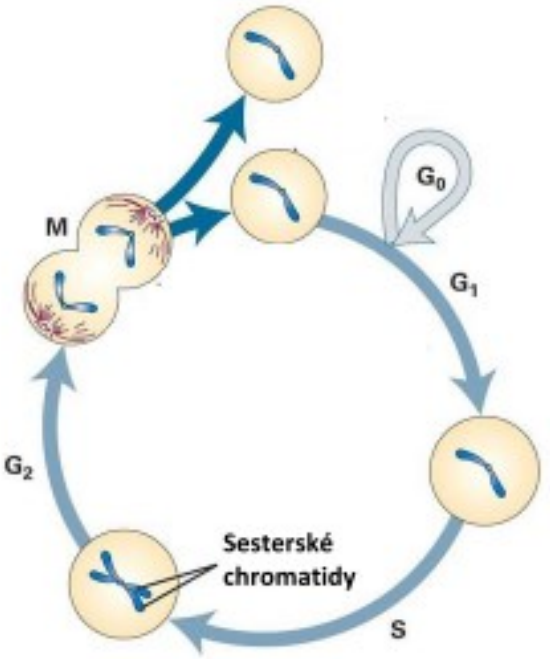




- G_0-phase (resting) maintained only basal metabolism
- It occurs only in certain cell types, especially those that have been terminally differentiated (neurons)

The synthesis of DNA during cell cycle

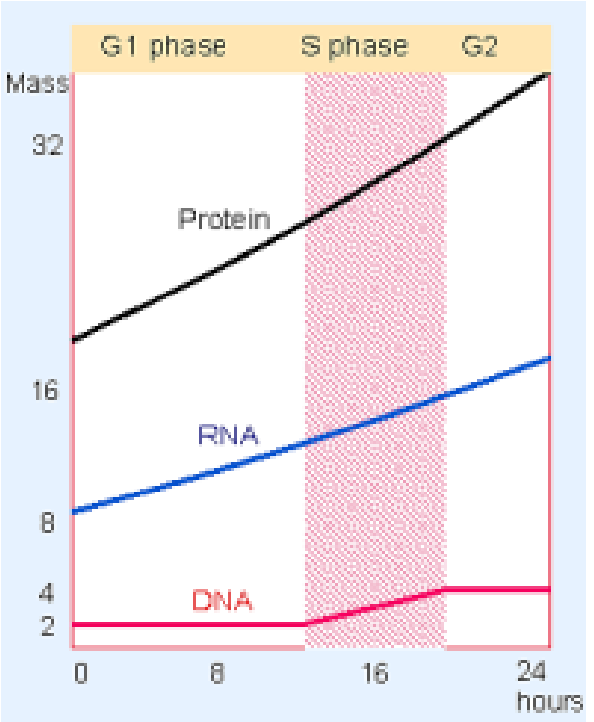
all structure and functions of cells are encoded by the DNA
• during cell division, each cell must obtain a complete genetic information
• DNA is replicated before the cell division
• there is a duplication of chromosomes
• cell in G2 phase has 2x higher DNA content than in the G1 phas
• resulting copies of chromosomes are joined at the **centromere** region, form **sister chromatids** which are separated during the nucleus division



Synthesis of proteins and RNA during the cell cycle

• still as fast during interphase
• in M phase the protein synthesis decreases and the synthesis of RNA is stopped

• all proteins are formed during interphase continuously
• exception are the histones, which are formed only in G1 and S phase
• After completion of replication, histone mRNAs are degraded



High accuracy requirements

- **correct sorting of phases**
- **flawless replication**
- **accurate chromosomes segregation**

Unwanted risks:

replication before mitosis: loss of genetic information at least in one cell

double replication of chromosomes before mitosis: increase of genes copy number

The length of the cell cycle is different

Time requirements of the cell cycle

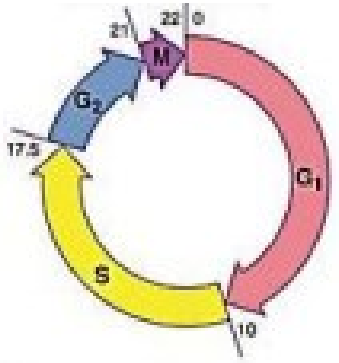
- the speed of the cell cycle is determined by **internal and external stimuli**
- cells with distinct specializations **without the ability to divide (neurons, muscle cells, red blood cells)**
- cells that divide **only under certain conditions (liver cells, lymphocytes)**
- **rapidly dividing cells (epithelial, blood stem cells)**

yeasts 1.5 - 3 hours, intestinal epithelium 12 hours, mammalian fibroblasts in culture for 20 hours, mammalian liver cells about 1 year

interphase **occupies most of the time**, can last for hours, days, weeks, months, years depending on the type of cells

- **proliferating human cell has about 22 hour cycle**
- cell that stops to diferentiate enters the resting phase G0
- variability is given by length of G1 phase

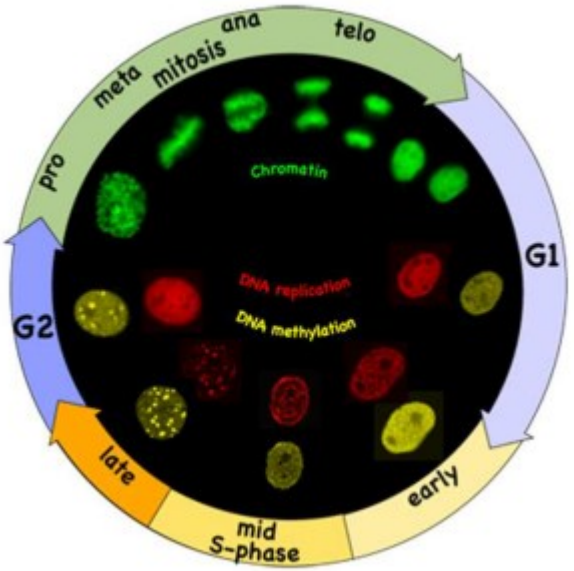
Cell type	Length of teh cell cycle
Yeast cells	1,5 – 3 hours
intestinal epithelium cells	12 hours
mammalian fibroblasts	20 hours
mammalian liver cells	1 year



Cell cycle phase	Length (hours)
G_1	10
S	7.5
G_2	3.5
M	1.0
Generation time	22

How is the accuracy of cycle achieved?

- **the use of checkpoints, which stop the beginning of each subsequent step before completing the previous step**
- damage of checkpoints by mutation: chromosomal rearrangements, abnormal numbers of chromosomes, changes in gene expression, etc. (cancer risk)
- **system of regulation of DNA replication and segregation of chromosomes is** in all eukaryotic cells identical
- similar to the transcription and translation: **the management system of the cell cycle is conservative and evolutionary stable**

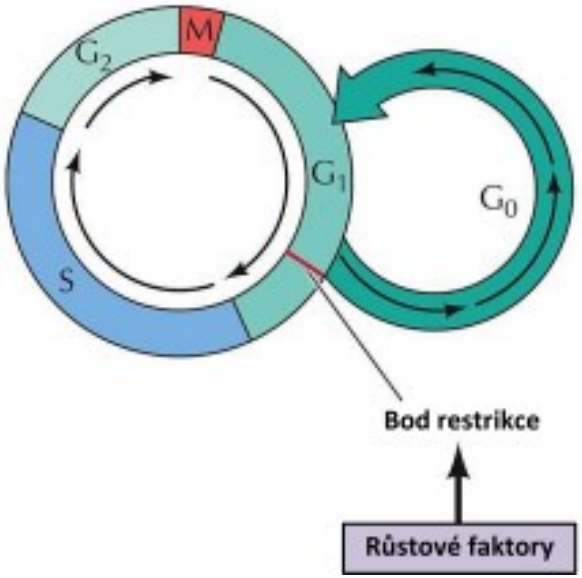


Restriction point

- in late G1 phase
- **for transition from G1 to S an extracellular signal is needed**

(growth factor, mitogen)

- **Without this signal cell leaves the cycle and enters the G0**



The cell cycle of eukaryotic cells

M-phase (mitotic)

- Mitosis (karyokinesis) = division of the nucleus
- cytokinesis = division of the cytoplasm

INTERPHASE

G_1-phase (presynthetic)

S-phase (synthetic)

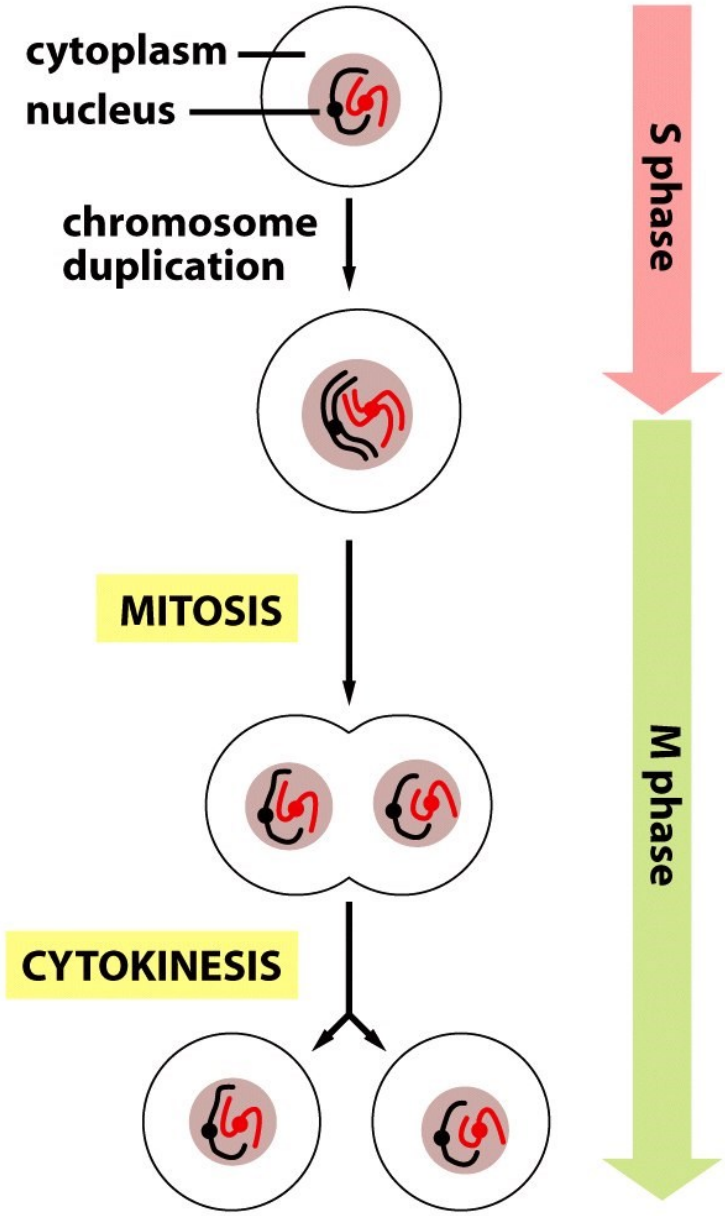
- replication of nuclear DNA
- synthesis of histones

G_2-phase (postsynthetic)

- protein synthesis, RNA

G_0-phase (resting)

- maintained only basal metabolism
- It occurs only in certain cell types, esp. those which are already terminally differentiated (neurons)



S –phase (synthetic)
The cell replicates nuclear DNA
- duplication of chromosomes

M–phase (mitotic)

Cell growth stops and is followed by

1) Nuclear division
   - condensation of chromosomes
   - formation of the mitotic spindle
2) Cytoplasmic division
   - formation of contractile ring
   - division of organelles

MT and ChL are divided,
GA and ER disintegrates into small fragments, which increases the probability of uniform distribution.

G1 phase

First growth phase after cell division, **follows the cytokinesis**

- cell needs to reach its original parent cell size
- in early embryos G1 and G2 phase is missing, the cell divides and diminishes

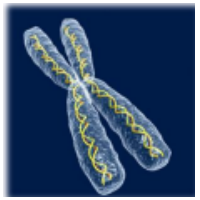
creation of cytoplasm and new organelles, synthesis of RNA, structural and regulatory proteins

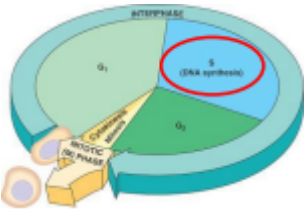
- **preparation of cells for S phase - synthesis of replication proteins and histones**
- **normal metabolic activity**
- length depends on the cell type, availability of nutrients and growth factors, the presence of inhibitors of proliferation
- In optimal conditions, 9-11 hrs
- the genetic material in the form of decondensed chromatin, 2n DNA molecules (46 in humans)
- **restriction point is sensitive to external factors, the first cell cycle checkpoint**
- At the end of the phase, the cell decides how it will proceed

  i) **entry into the G0 phase**: resting phase, cell does not divide
  the absence of growth factors, senescence, differentiated cells

  ii) **entry into S phase**: after overcoming the restriction point, the cell continues by next cell division

S phase





semi-conservative DNA replication

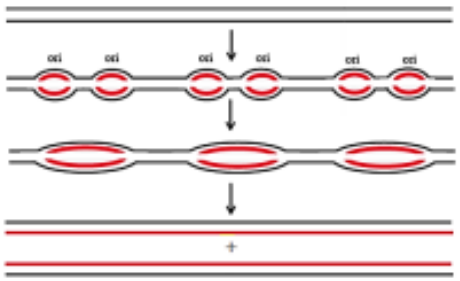
• within the **replicon** based in places ori bidirectional replication fork

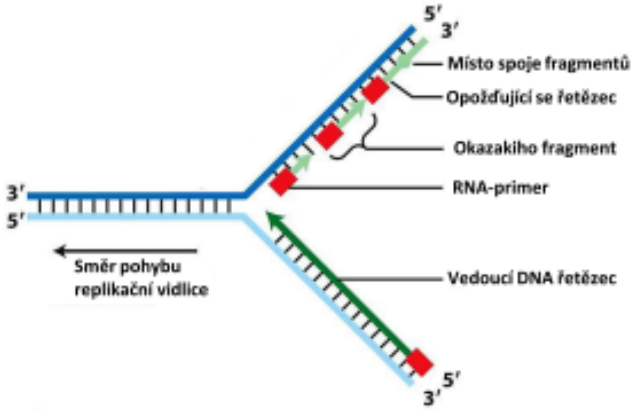
• **replication proteins - initiating proteins, helicases, polymerases, RNases, ligases, telomerase**

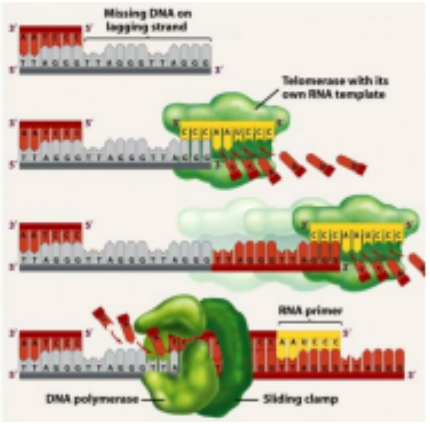
• DNA synthesis requires a template DNA strand and the RNA primer

• leading strand is formed continuously lagging strand is formed through Okazaki fragments

• replication of the ends of linear chromosomes is ensured by **telomerase**



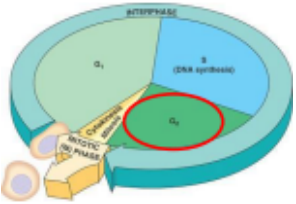




correctness of replication is ensured by **accuracy and proofreading DNA polymerase activity, error base pairing repair**

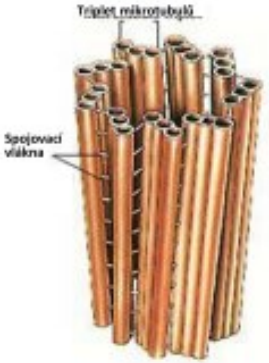
• duplication of genetic material, chromosomes composed of sister chromatids

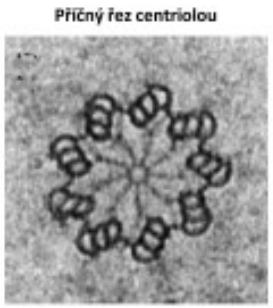
G2 phase

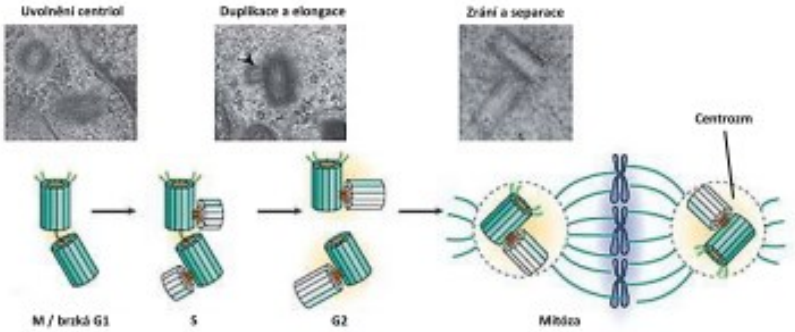


second growth phase, premitotic phase

- protein synthesis and formation of organelles, systematic preparation for M phase
- synthesis of **proteins of mitotic spindle, centriole duplication**
- replicated genetic material, 2 x 2n DNA molecules, two sister chromatids in chromosome
- **2nd cell cycle checkpoint, checking the integrity and accuracy of DNA replication**







Centriole

- paired organelle of eukaryotic cell, not in plant and fungi cells
- capable of independent division
- nine microtubule triplets around the central cavity
- two connected perpendicularly oriented in M and G1 phase
- duplication in S and G2 phase, in the resulting pair there is always one original centriole and one new
- together with its surroundings form **the centrosome**, **which organizes the mitotic spindle**

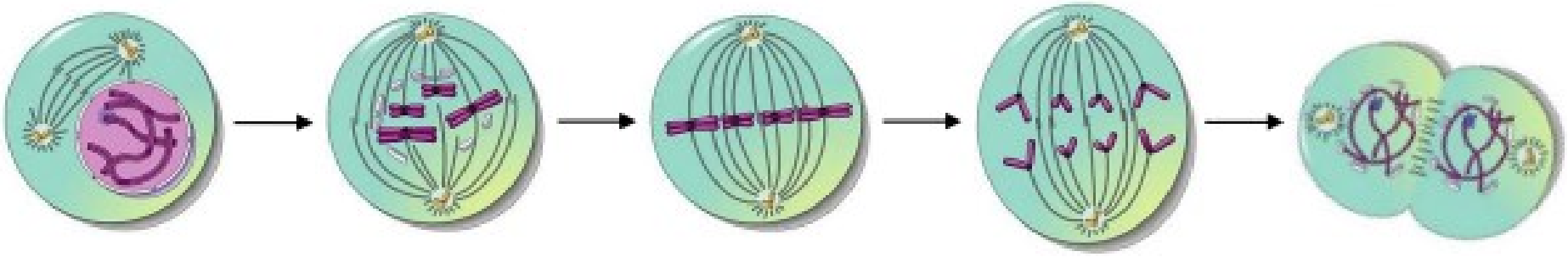
M phase

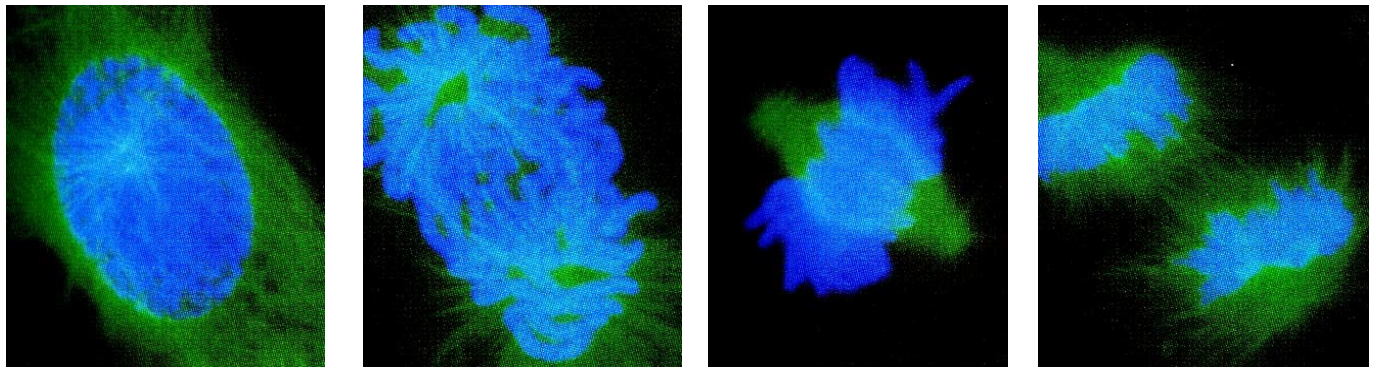
It includes nukleus division (mitosis, karyokinesis) and the cytoplasm (cytokinesis)

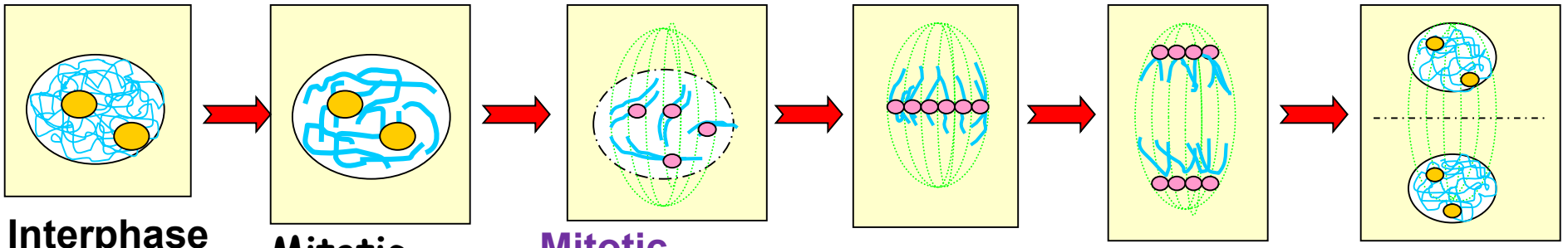
MITOSIS

- based on changes in **morphology and behavior of chromosomes**

mitosis is divided into 5 phases



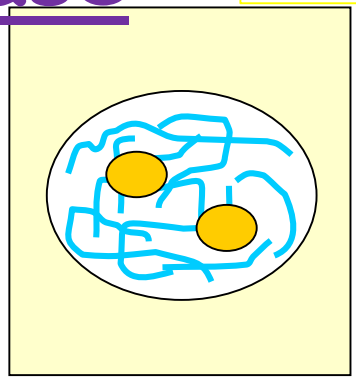


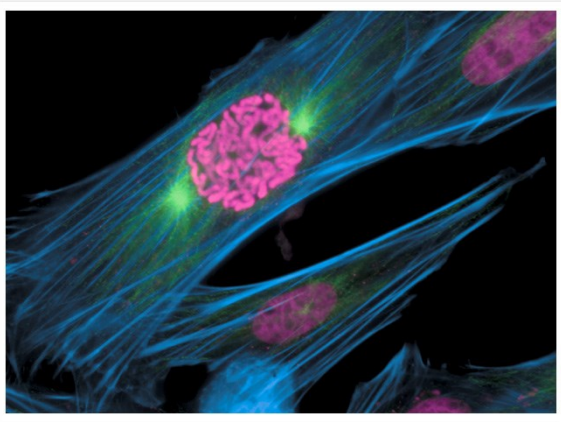


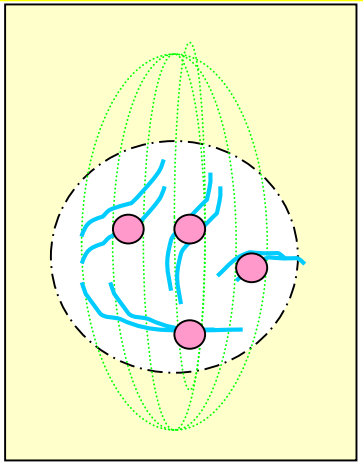
M-phase

Prophase a prometaphase

chromosome condensation occurs







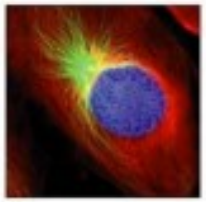
Outside the core begins to rise the mitotic spindle

Early mitotic prophase

Late mitotic prophase (prometaphase)

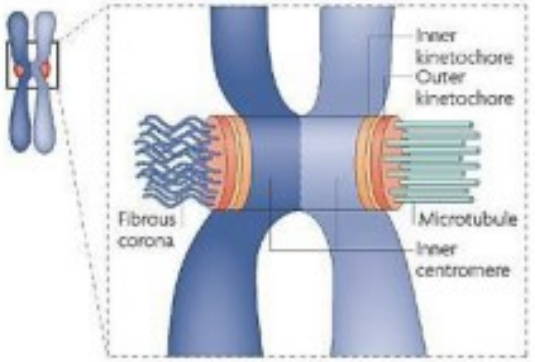
1) Prophase

- condensation of chromosomes to form visible under light microscope
- **sister chromatids connected in centromeric region**
- **centrosome shift to opposite sides of the cell**
- the formation of the mitotic spindle begins in centrosomes



2) Prometaphase (late prophase)

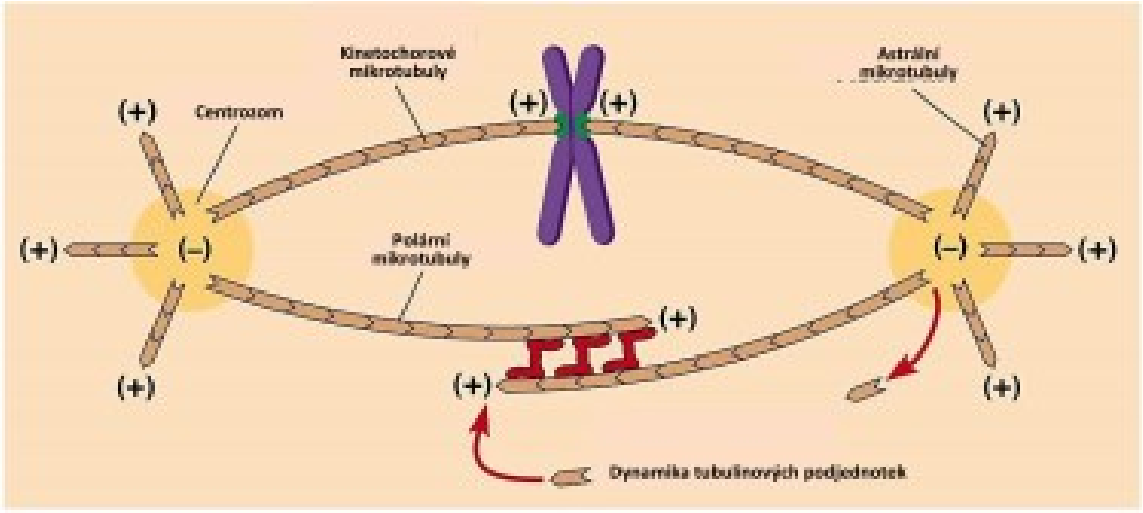
- **formation of the kinetochores on chromosomes**
  - A large protein complex in centromeric region
  - Allows to bind chromosomes to spindle microtubules
  - Inner layer recognizes and binds to centromere DNA
  - An intermediate layer connects the outer and inner layer
  - The outer layer is connected to the (+) ends of microtubules, contains motor proteins, based on the fibrous corona

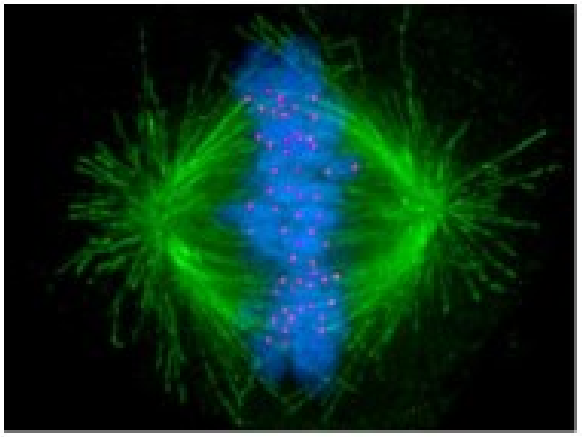


M-phase prometaphase

disintegration of the nuclear membrane and nucleolus

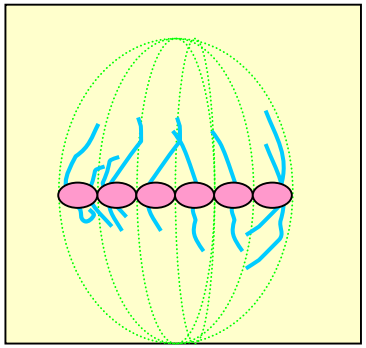
- stabilization of centrosomes position on opposite sides of the cell
- movement of chromosomes into the center of the cell
- **formation of the mitotic spindle**
  - Tubulin polarity: (-) end in the centrosome, (+) end grows at the opposite end
  - Dynamic structure, shortening and lengthening (de) polymerization of tubulin
  - After the disintegration of the nuclear membrane (+) the ends of the spindle contact the kinetochore
  - Kinetochore microtubules: connected to kinetochores
  - Polar microtubules: connected to tubules from the opposite pole of the spindle
  - Astral microtubules: attached to the plasma membrane proteins

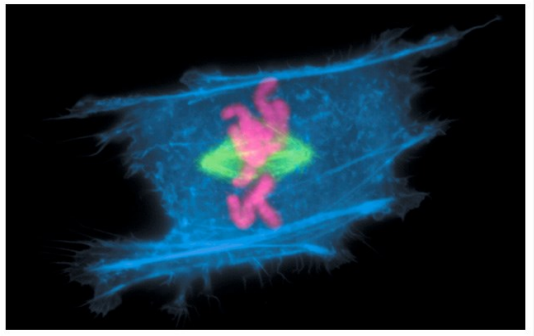




M-phase Metaphase

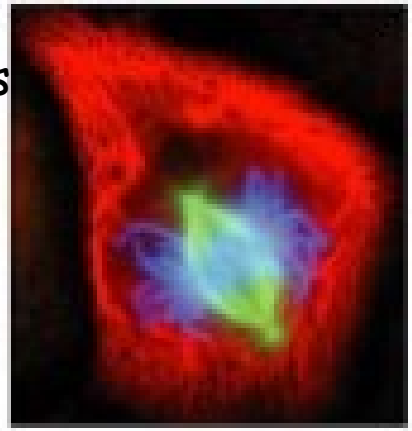
Chromosomes are grouped in the equatorial plate and form a metaphase plate.



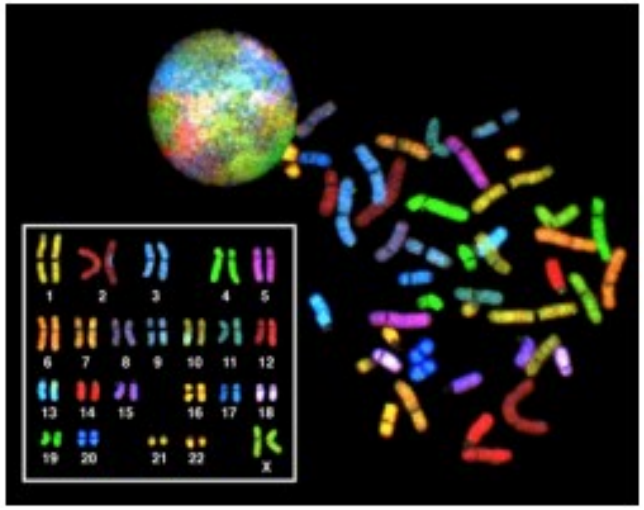


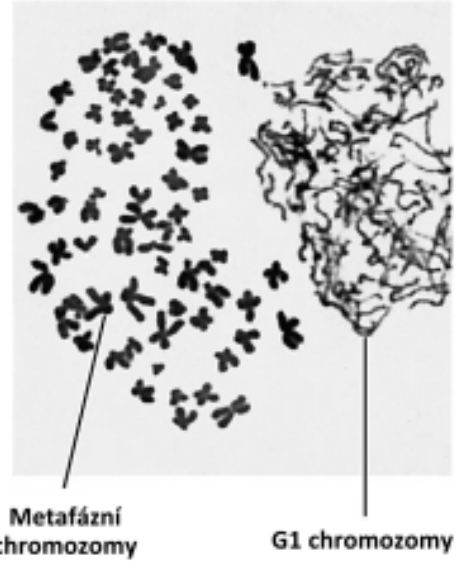
Chromosome condensation is completed

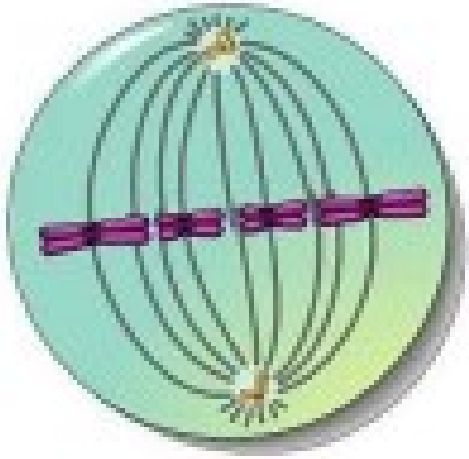
- chromosomes are pulled / pushed by kinetochore microtubules
- sorting of chromosomes in the equatorial plate between the poles of the mitotic spindle **(metaphase plate)**
- sister chromatids of each chromosome are connected to opposite poles of the spindle
- establishing the karyotype from the cells which were stopped in metaphase (colchicine)



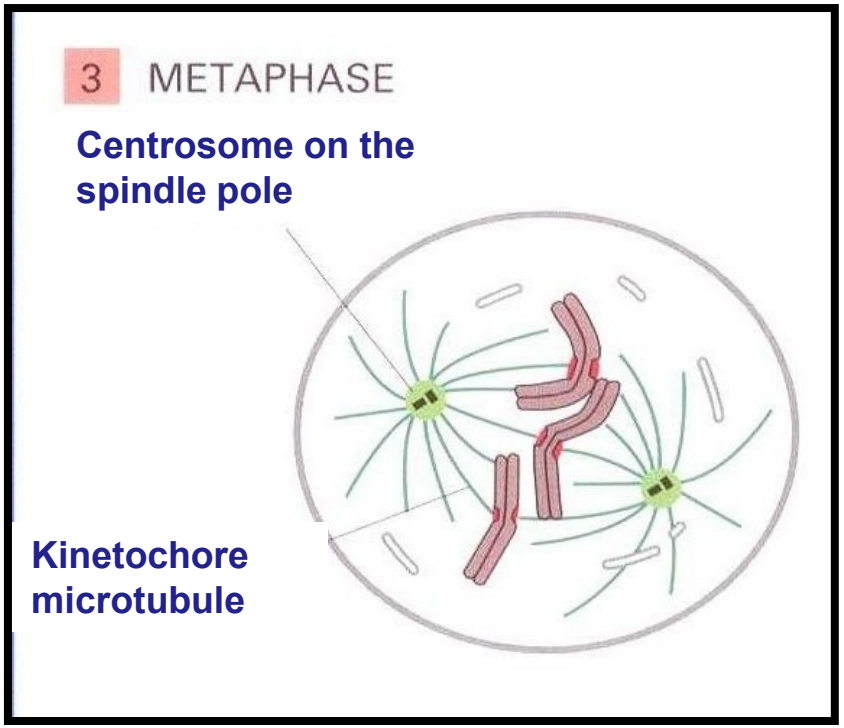
Spektrální karyogram







Metaphase

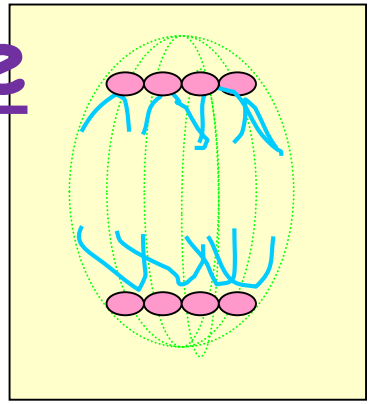


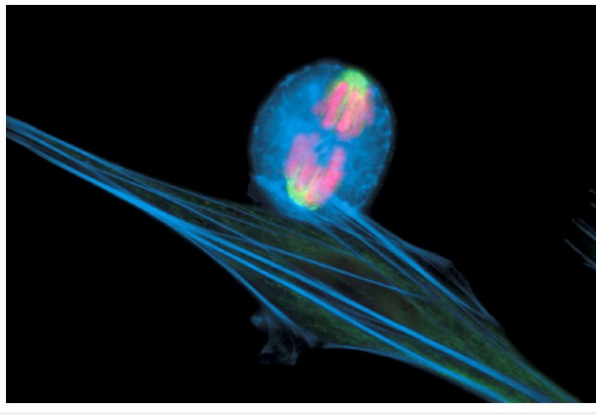
1. **Metaphase beginning is defined by formation of metaphase plate.** Chromosomes are aligned in the **equatorial plane** midway between the poles. Kinetochores of all chromosomes are also aligned in a plane.

2. Chromosomes in metaphase plate are held with considerable force.

Microtubular molecular motors **(motor proteins)** and a gradual build-up and breakdown of microtubules are involved in the establishment and maintenance of this state. Tubulin units are either added or deleted, which leads to movement.
Colchicine = mitotic spindle poison, blocks addition of microtubule subunits

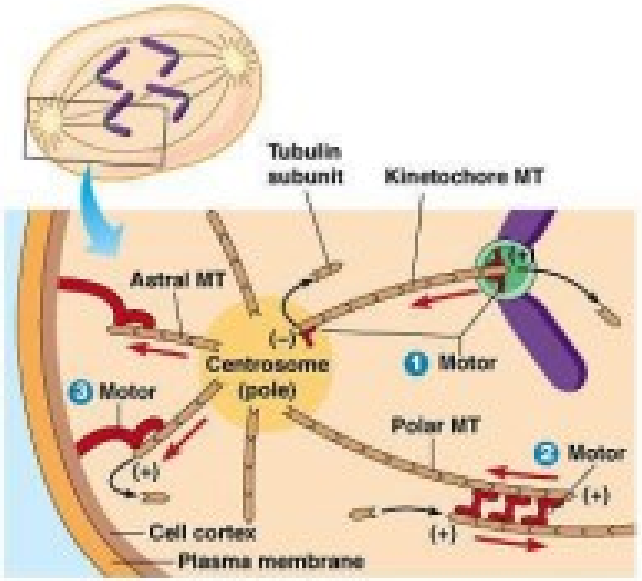
M-phase Anaphase

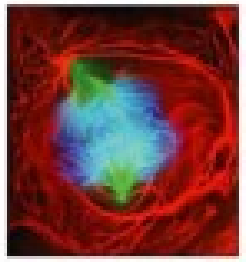


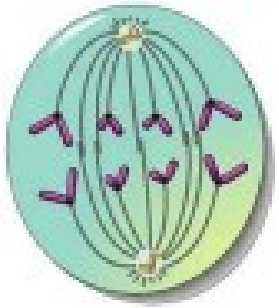


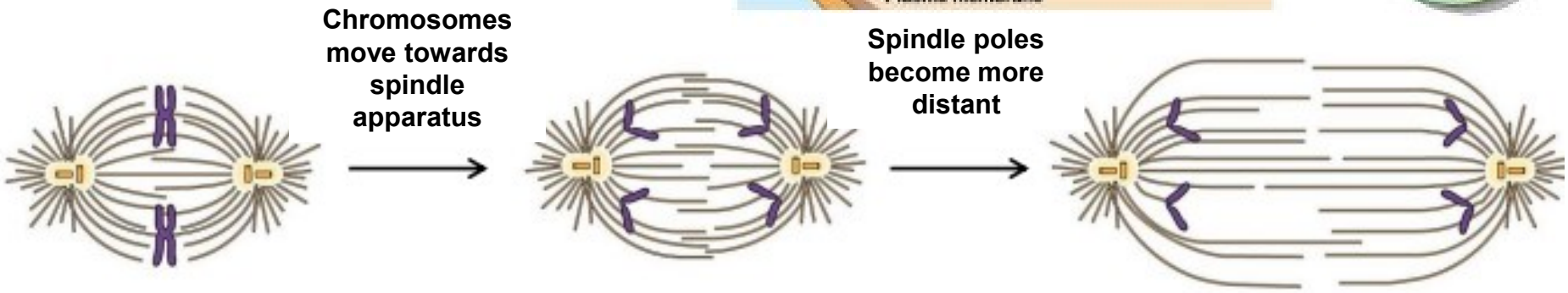
Sister chromatids split and migrate towards opposite ends of the cell

- chromosomes separate at centromeres, sister chromatids split
- chromatids move towards spindle
  - kinetochore microtubules shorten
  - spindle poles become more distant
  - participation of motor proteins
- the same set of genetic information is assembled at each spindle pole, establishing a base for daughter cells

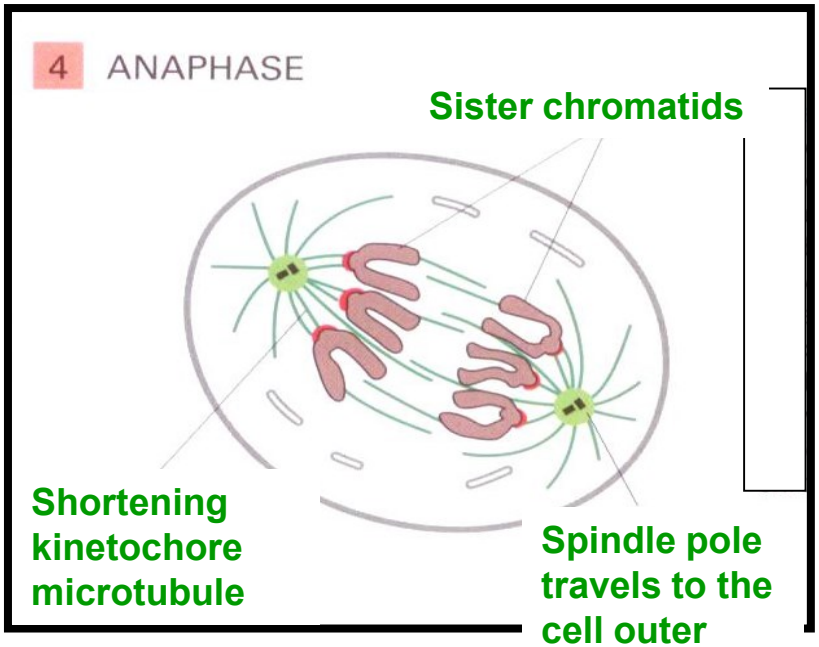








Anaphase



1. The connection between sister chromatids is broken by proteolytic enzymes.

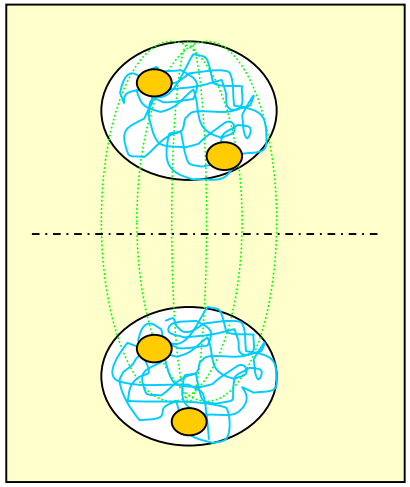
Each chromatid (daughter chromosome) is pulled toward the spindle pole, to which it is connected.

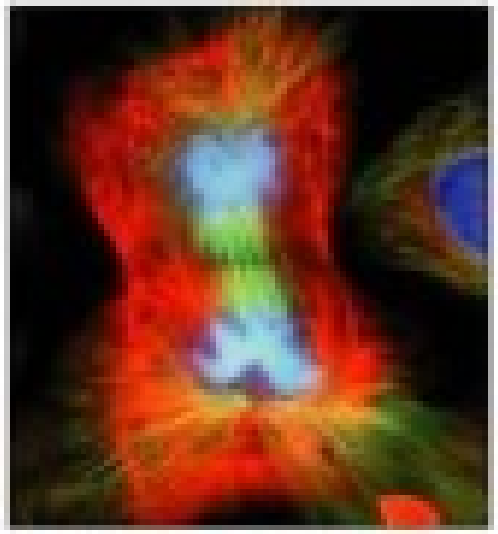
2. This segregation of chromosomes leads to a chromosome distribution into two identical sets at opposite ends of the cell.

The chromosome movement speed is 1μm per minute. The movement speed is the result of two independent processes (anaphase A - anaphase B)

M-phase Telophase

New nuclear envelope is created around each set of chromosomes to produce two daughter nuclei

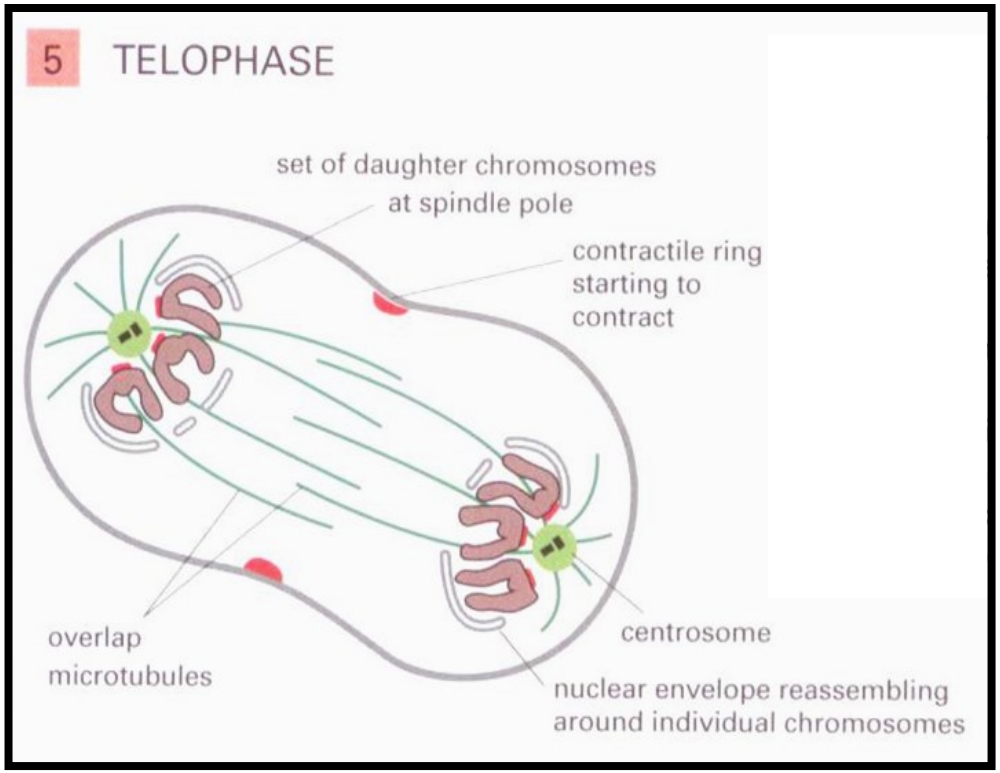




Polar microtubules continue to lengthen, **preparing for cytokinesis**

- chromosomes, which arrived to the poles of the spindle begin to decondense
- kinetochores and mitotic spindle deteriorate, new nuclei forms
- nuclear membrane restore around each set of sister chromatids

Telophase



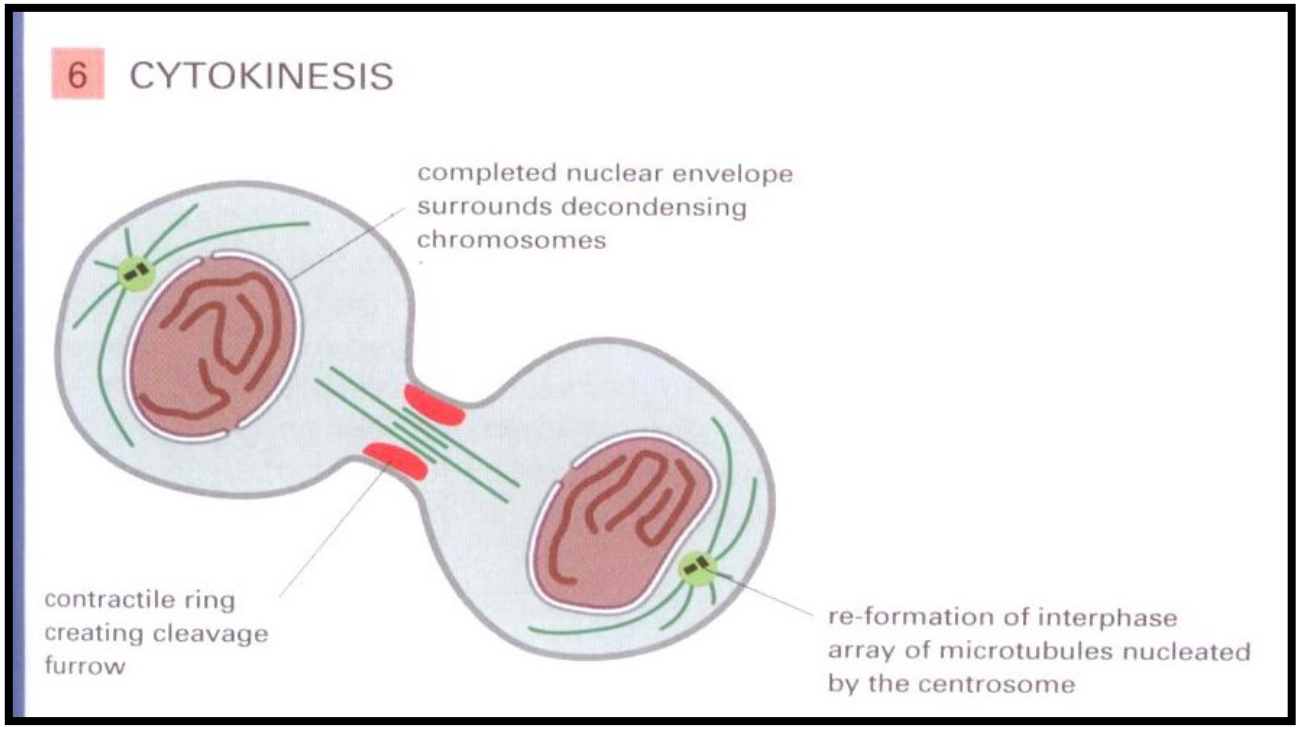
A new nuclear envelope begins to assemble around each set of chromosomes, producing two daughter nuclei.

Nuclear membrane vesicles gather around individual chromosomes and later fuse together, establishing nuclear envelope. Intermediate filaments, which were phosphorylated in prometaphase, are now dephosphorylated and associate back into **nuclear lamina**, which is under the nuclear envelope (inner x outer nuclear membrane)

Nuclear proteins penetrate through the freshly established nuclear membrane and the nucleus continues to grow.

Chromosomes decondense into so called interphase state, re-enabling transcription. Mitosis ends.

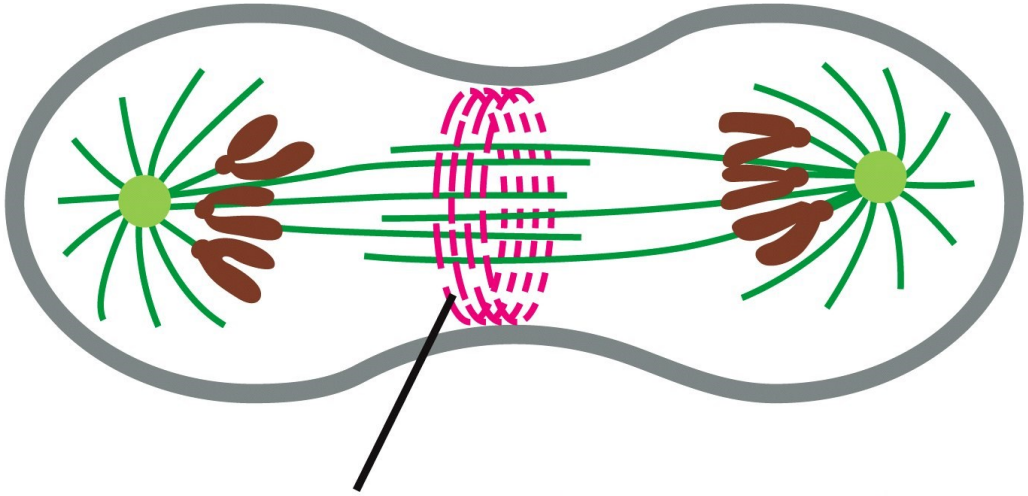
Cytokinesis



Cytokinesis is a division of cytoplasm and all of its components. Begins already in anaphase – cleavage furrow is created perpendicular to the mitotic spindle division axis. Contractile ring appears in anaphase as well.

Contractile ring is formed by bundles of actin and myosin filaments. It is attached to proteins associated with the inner side of the membrane and is able to develop a considerable force.

Actin filaments movement against myosin fibers is similar to muscle contraction. The contractile ring structure is however only temporary !! It disappears.

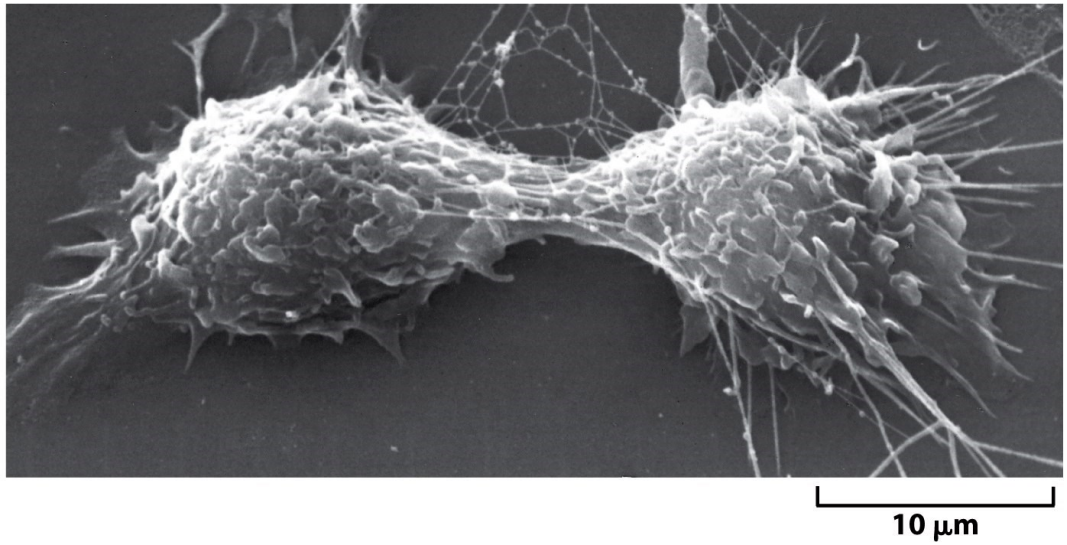


actin and myosin filaments of the contractile ring

cytokinesis does sometimes not follow immediately after mitosis - **SYNCYTIUM**

(polynuclear cell), is thus created

In cytokinesis, cell membranes are being formed at both daughter cells simultaneously = **CELLULARISATION**



Cells in animal tissues usually adhere firmly to their neighbors, they tend to be rather flattened and adhered to some sort of base.

Once the cell enters M phase, **integrin's** (responsible for holding the cells together in tissues) start to phosphorylate weakening their interactions and bonds, leading to cell rounding.

After cytokinesis finishes the cells reintroduce their adhesion forces and flatten once again. Reorganizing the contacts with neighboring cells allows for proper cell incorporation into tissue.

Meiosis

Asexual reproduction

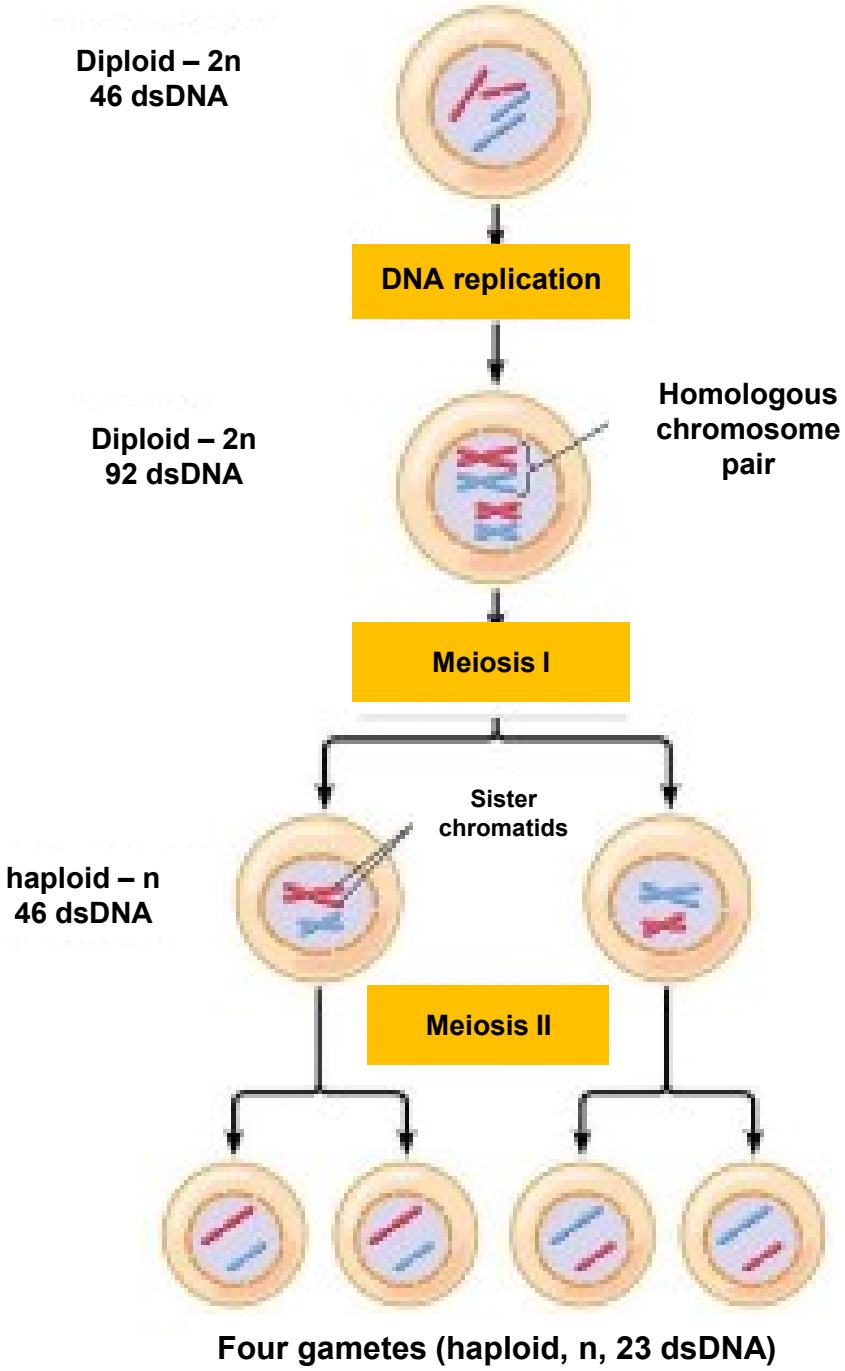
- cell divides to produce two new identical daughter cells
- e.g. **mitosis, binary fission**

Sexual reproduction

- two cells fuse to produce a new cell (zygote), that is different from original cells

Meiosis

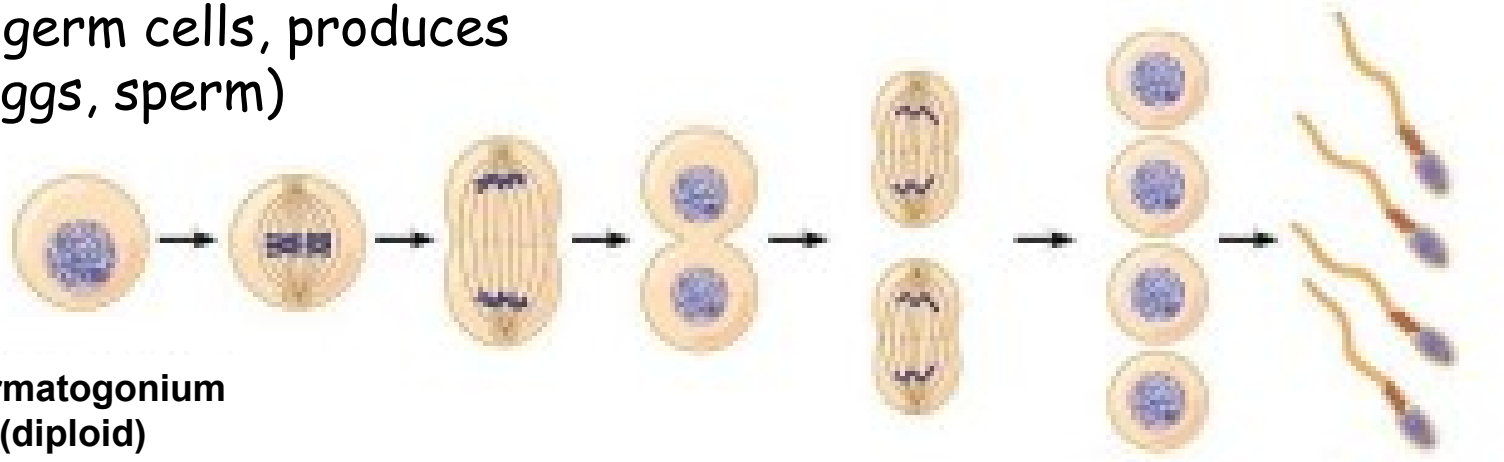
- reduction division (chromosomes)
- occurs in germ cells, produces gametes (eggs, sperm)
- chromosomes replicate in interphase, there are two meiotic divisions
- the original cell is diploid (2n) and produces four daughter haploid (n) cells
- chromosome number is reduced by half, fertilization restores the diploid status



Meiosis

- occurs in germ cells, produces gametes (eggs, sperm)

Spermatogenesis

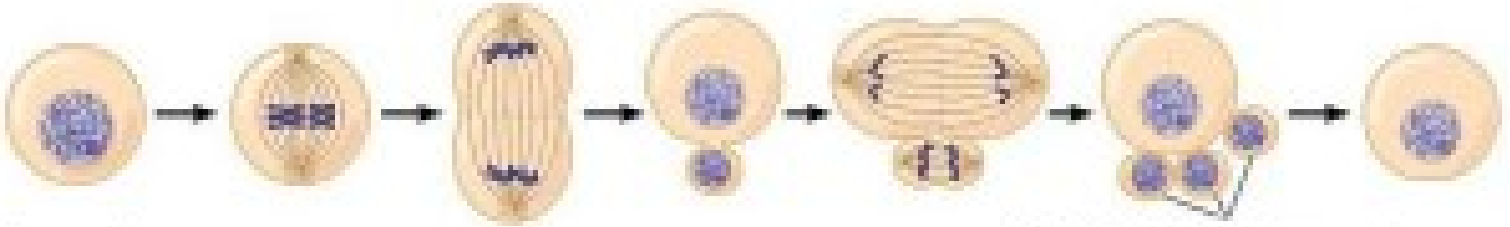


Spermatogonium (diploid)

Spermatids (haploid)

Sperms (haploid)

oogenesis



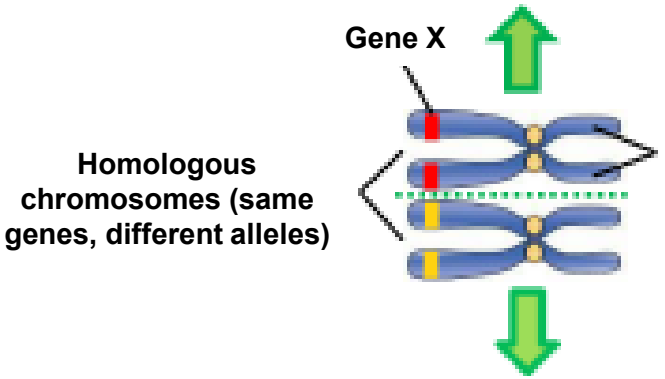
Oogonium (diploid)

Polar bodies (haploid)

Oocyte (haploid)

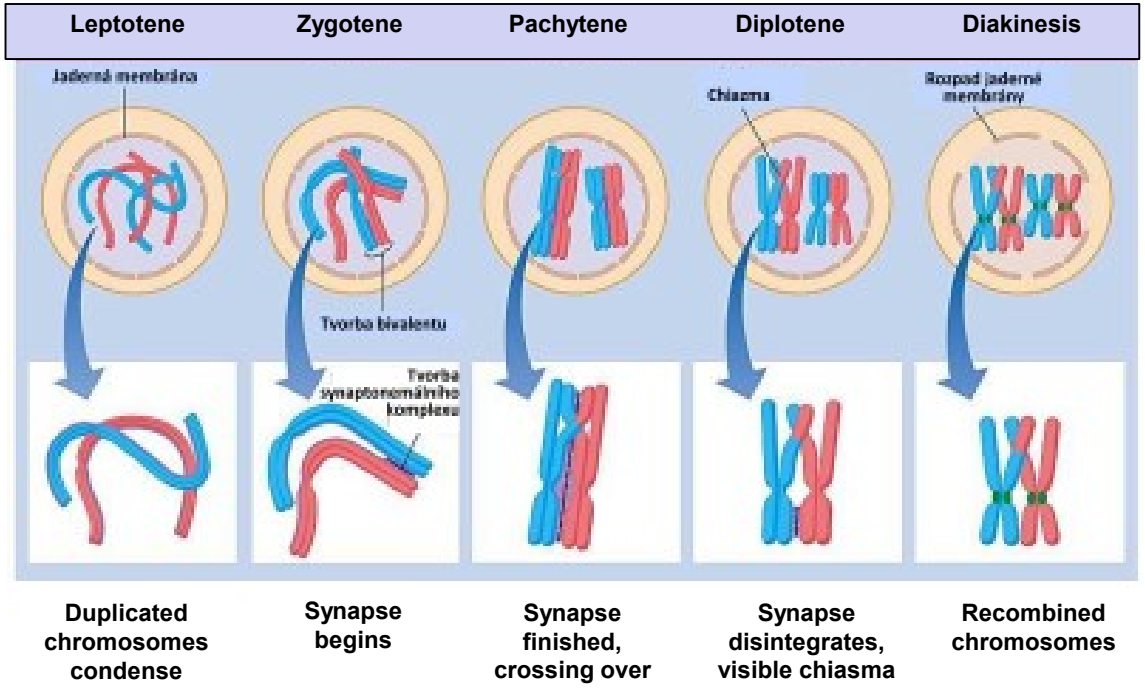
Meiosis I – reduction division

- homologous chromosomes with different alleles for the same genes separate
- unusual cell division type, that creates haploid cells with chromosomes originating from sister chromatids



Prophase I

- homologous chromosomes pair up, bivalent assembly (2 chromosomes), tetrads (4 chromatids)
- **crossing-over (homologous recombination)** takes place between homologous chromosomes connected by a synapse
  - genetic recombination in offspring is ensured by replacing parts of chromosomes
- chromosomes condense, spindle apparatus forms, nuclear membrane disintegrates
- consists of five phases



Meiosis I – reduction division

Metaphase I

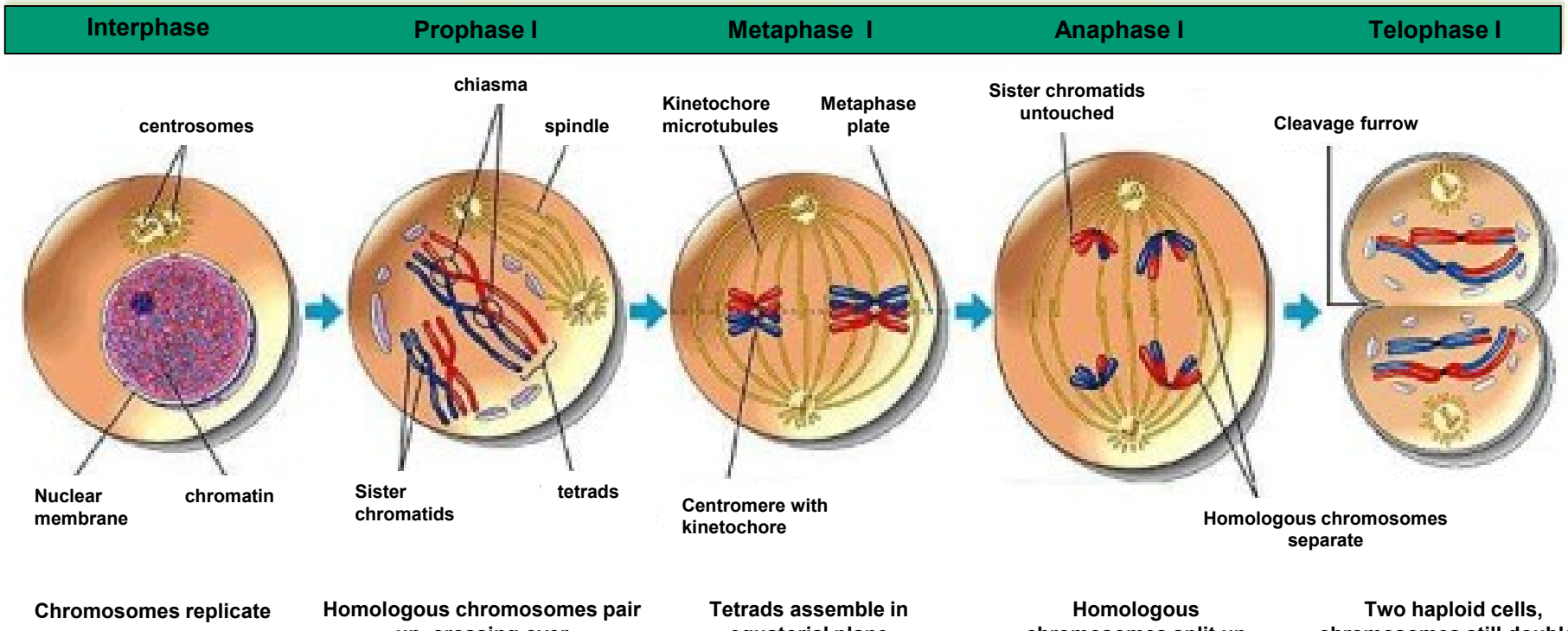
- chromosome homologous pairs assemble in equatorial plane

Anaphase I

- homologous chromosomes separate and move towards spindle apparatus
- sister chromatids remain connected by centromeres

Telophase I

- spindle apparatus disintegrates, nuclear membrane restores
- cytokinesis splits the cell into two



Meiosis II – separation division

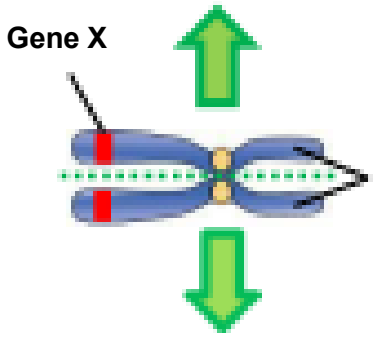
- just one chromosome homologue is present in cell after meiosis I
- during meiosis II sister chromatids, that contain matching information separate into newly forming gametes

Prophase II nuclear membrane disintegrates, spindle apparatus forms

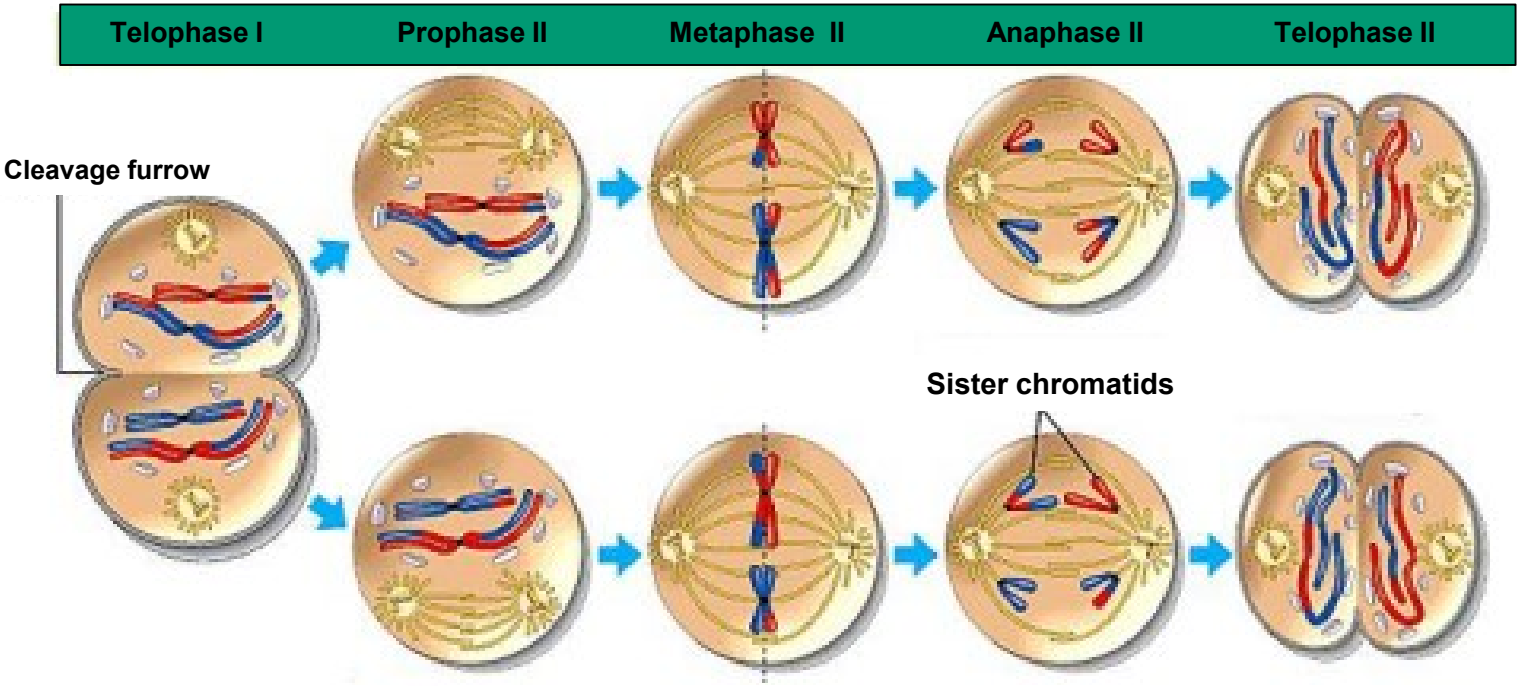
Metaphase II chromosomes align in equatorial plane

Anaphase II sister chromatid separation and their movement towards opposite poles of spindle apparatus

Telophase II spindle apparatus disintegrates, nuclear membrane restores, cytokinesis



Sister chromatids (same genes, same alleles)



Two haploid cells, chromosomes still doubled

Sister chromatids split during meiosis II. Four haploid cells containing a copy of each chromosome arise.

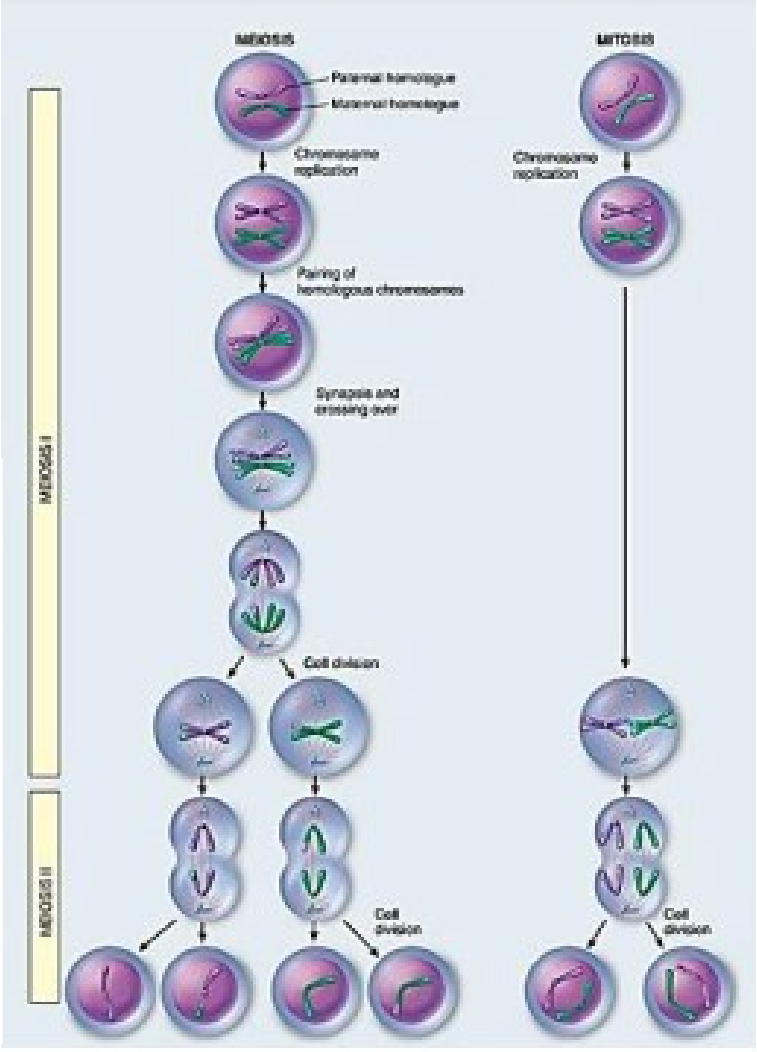
Meiosis

outcome in four haploid cells, each with a copy of every chromosome

- **crossing-over generates altered allele combinations** within chromosomes
- meiosis is the basis for sexual reproduction, depends on sex chromosome presence
- **offspring produced by sexual reproduction is genetically different from its parents and siblings**

- sexual reproduction is a source of genetic diversity stemming from:
  i) unbiased chromosome choice: chromosomes split randomly in meiosis I
  ii) random fertilization: random fertilization of an egg by a sperm resulting in zygote
  iii) crossing-over: new allele combinations coming from parent chromosomes

	Mitosis	Meiosis
DNA replication	During interphase, before mitosis	During interphase, before meiosis, only once
Number of divisions	1	2
Homologous chromosome linked by a synapse	Does not occur	Occurs together with crossing-over between non-sister chromatids in prophase I
Daughter cell number and their genetic content	Two diploid (2n) daughter cells, genes identical with parent cell	Four haploid (n) daughter cells, genes different from parent cell
Role in human body	Growth, repair	Genetic diversity in sexual reproduction



Regulating cell cycle

decision whether to split is a **cellular reaction to its size and extracellular signals (nutrients, growth factors, stress factors, DNA damage)**

- yeasts decide mainly according to cell size and nutrient availability
- mammals adjust according to **growth hormones and mitogens**

Principles in cell cycle regulation

- mitosis wont happen before DNA replication finishes, DNA replication wont happen without splitting the cell
- damaged DNA must not replicate and be passed onto daughter cells
- erroneously paired chromosomes must not finish mitosis
- cell cycle deregulation can lead to carcinogenesis

Healthy tissue: organized cell growth

cancer: uncontrolled cell growth

Cell cycle regulation elements

Foundation of this regulation lies in **specifically activating proteins** that regulate cell cycle

i) Posttranslational protein modifications are a part of cell cycle regulations

- **phosphorylation: major change to protein structure and function, reversible**

  proteinkinases are among the main cell cycle regulators

  kinases of the CDK group, cyclines, cyclin inhibitors

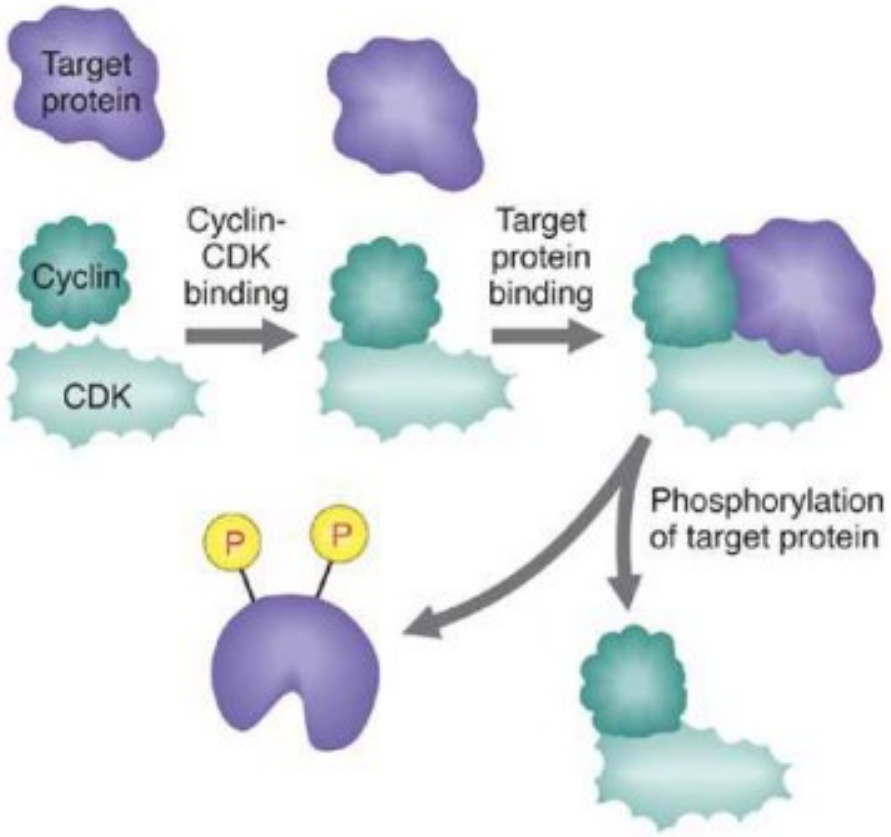
- **proteolysis: unneeded proteins are labelled by ubiquitin and decomposed in proteasome, irreversible degradation system**

ii) Cell cycle checkpoints

CDK – cyclin dependent kinases

heterodimer proteinkinases with a regulatory (cyclin) and catalytic (kinase) component (cyclindependent kinase, CDK)

They phosphorylate key regulator proteins and are thus involved in cell cycle management



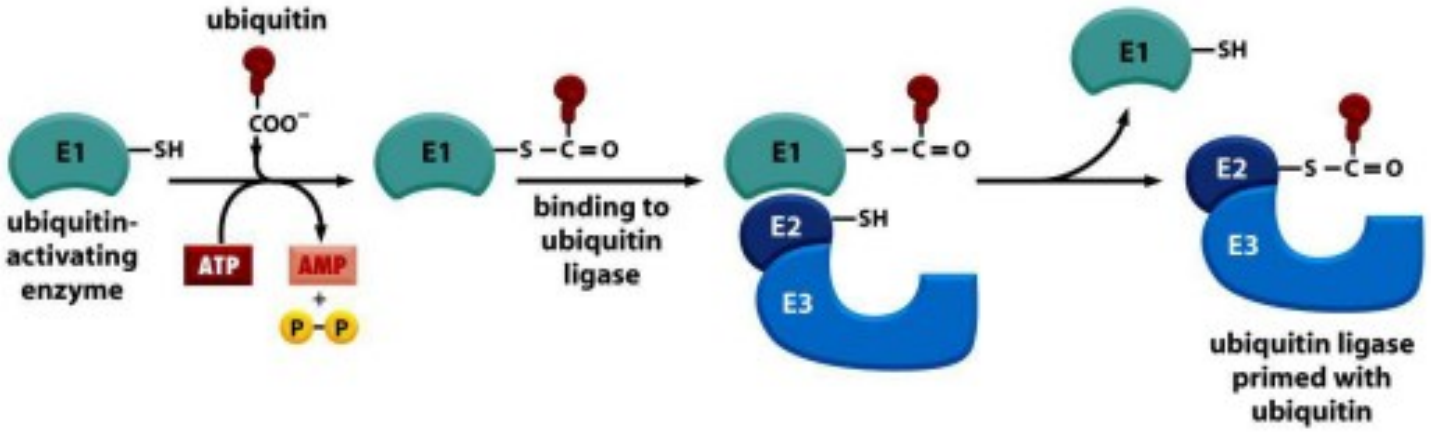
Systems for protein degradation:

- **targeted degradation of proteins regulating cell cycle, results in unidirectional irreversible cell phase shift**
- **Ubiquitin ligase:**
- **SCF** is a multi-protein E3 ubiquitin ligase complex catalyzing the ubiquitination of proteins destined for proteasomal degradation. It has important roles in the ubiquitination of proteins involved in the cell cycle and also marks various other cellular proteins for destruction
- **„anaphase promoting complex" = cyclosome (APC/C)**
- **Ensures labelling targeted protein by polyubiquitin mark, that predetermines proteins for degradation in proteasome**

Ubiquitin-proteasome system

Ubiquitin activation

- Catalyzed by ubiquitin-activating enzyme E1
- ATP dependent reaction
- Ubiquitin is attached by C-terminus to E1 protein
- Ubiquitin is consequently carried over from E1 onto ubiquitin-conjugating enzyme E2
- Enzyme E2 works in conjunction with E3 enzyme, this complex is termed ubiquitin-ligase

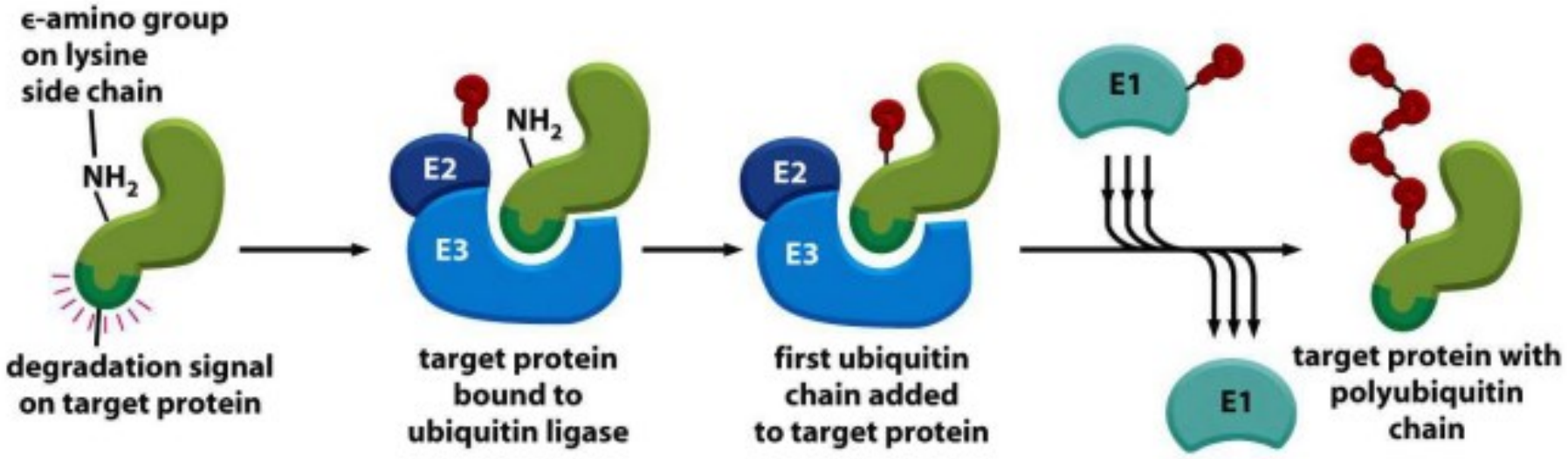


Proteasome degradation: major targets

- **Cell cycle and growth regulators:**
- Components of cell signal cascades
- transcription factors
- metabolism enzymes
- protein defective variants resulting from mutations or faulty proteosynthesis
- major histocompatibility complex I antigens

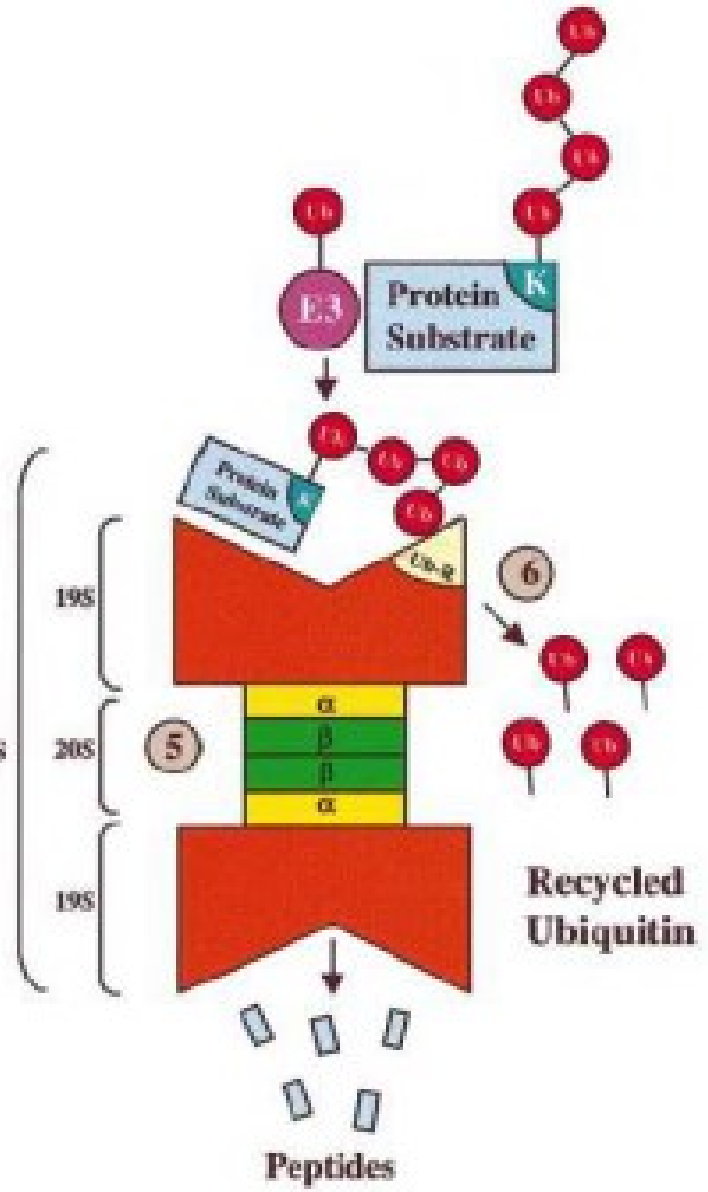
- Proteasome inhibition is lethal to the cell

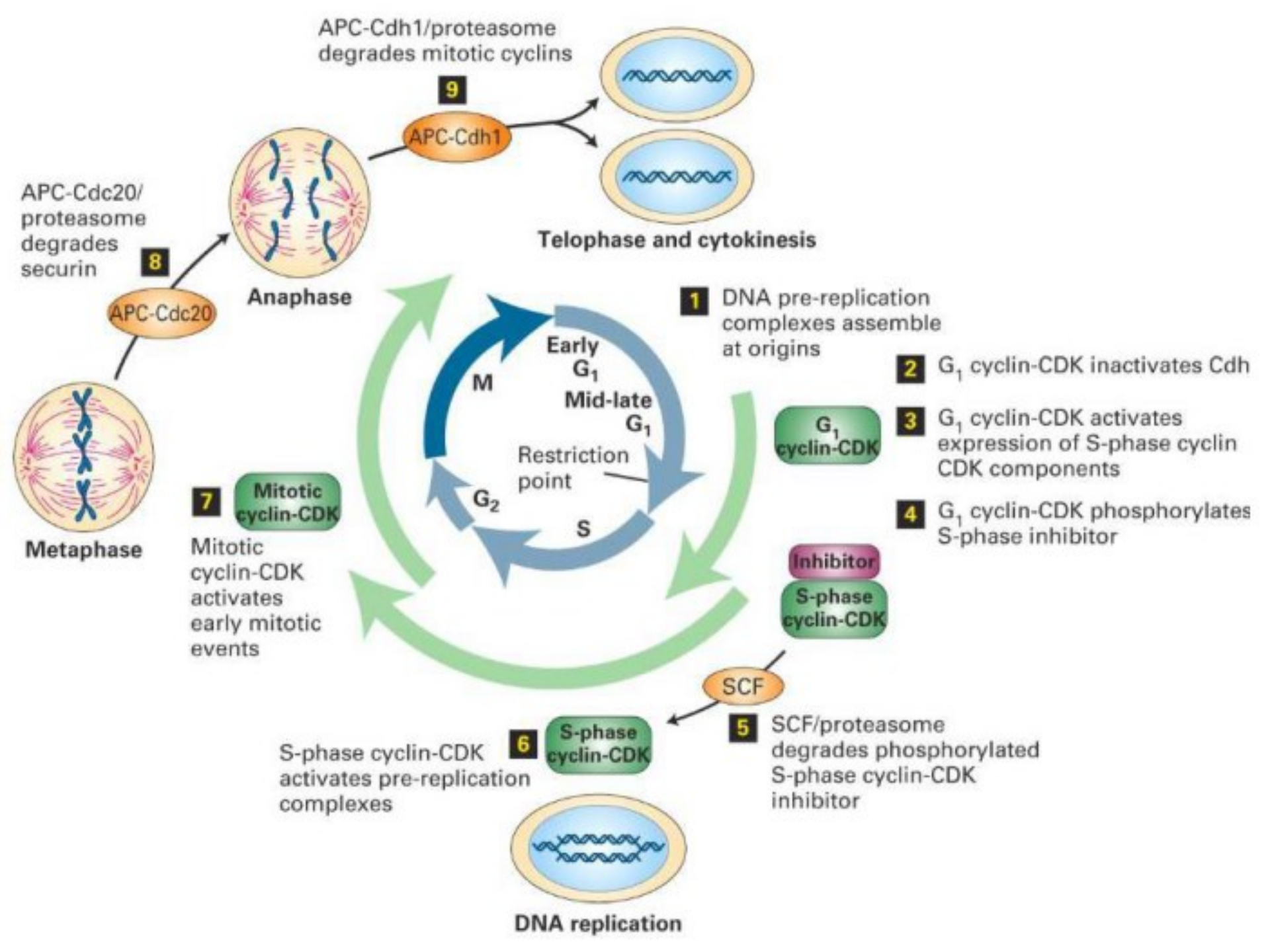
- Mammals posses hundreds of ubiquitin-ligases, that recognize **degrons** (signals for degradation) on target proteins by recognition section E3
- Ubiquitin-ligases then manage the ubiquitination itself, producing polyubiquitin chains attached in several steps to target protein or previous ubiquitin at lysine residue



Degradation in the proteasome

- Binding of poly-Ub labeled substrate to Ub receptor subunit of the proteasome
- Cleavage of Ub through izopeptidase bound to hatch
- substrate translocation to the proteasome column
- degradation of the substrate into small peptides




APC-Cdh1/proteasome degrades mitotic cyclins
9
APC-Cdh1
Telophase and cytokinesis
APC-Cdc20/ proteasome degrades securin
8
APC-Cdc20
Anaphase
Metaphase
1 DNA pre-replication complexes assemble at origins
2 G1 cyclin-CDK inactivates Cdh
3 G1 cyclin-CDK activates expression of S-phase cyclin CDK components
4 G1 cyclin-CDK phosphorylates S-phase inhibitor
G1 cyclin-CDK
Inhibitor
S-phase cyclin-CDK
SCF
5 SCF/proteasome degrades phosphorylated S-phase cyclin-CDK inhibitor
6 S-phase cyclin-CDK
S-phase cyclin-CDK activates pre-replication complexes
DNA replication
7 Mitotic cyclin-CDK
Mitotic cyclin-CDK activates early mitotic events
Early G1
Mid-late G1
Restriction point
S
G2
M

Cyclin-dependent protein kinases (CDK)

- key enzymes which regulate the transition between cell cycle phases, transfer cells from the G0 to G1
- various phases ofCC BC triggered by phosphorylation of specific target proteins
- their activity is strictly regulated

i) Catalytic activity only in complex with cyclin

ii) Tyr phosphorylation sites – activating, inhibitory

- phosphorylation by specific kinases, dephosphorylation by phosphatases

iii) inhibitors

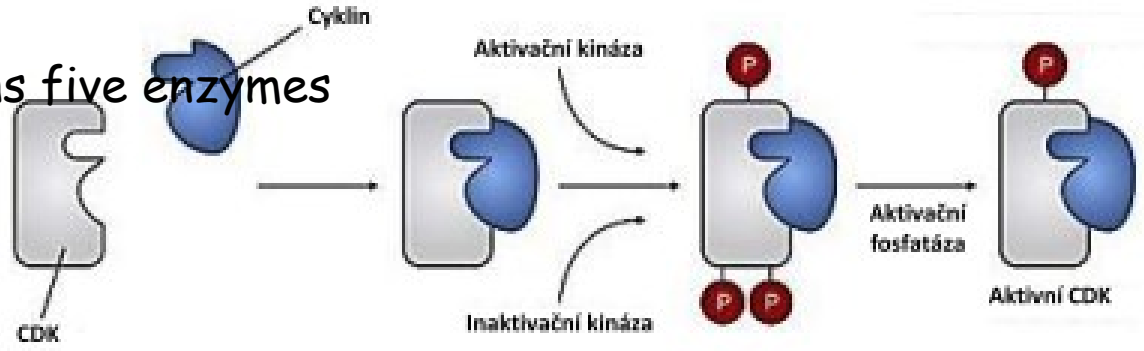
- in yeast one enzyme, in humans five enzymes

G1 CDK4, CDK2, CDK6

S CDK2

G2 CDK2, CDK1

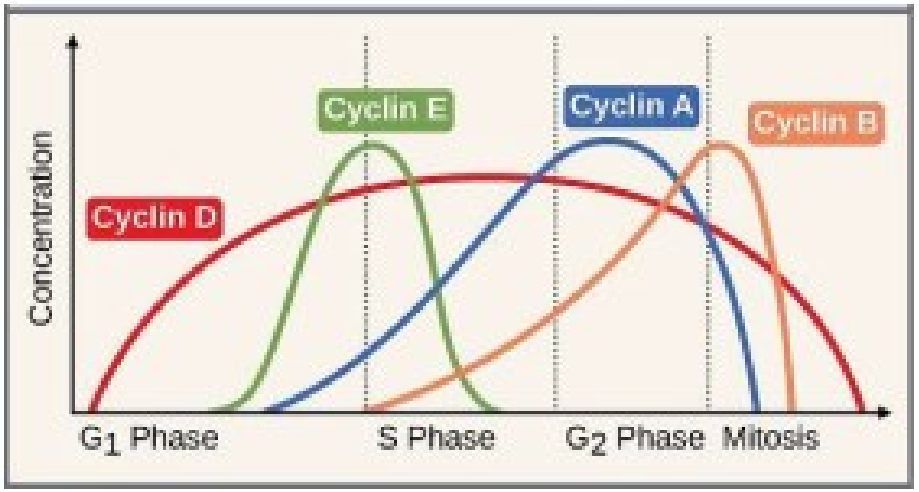
M CDK1

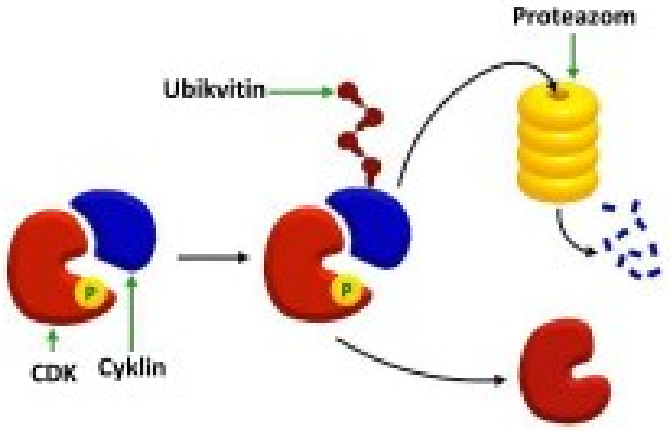


- negative regulators of CC passage, two families (INK, CIP)
- They bind to CDK and inhibits their activity, responsible for the arrest in G1
- e.g. P21 is actively synthesized during DNA damage, binds to CDK and interrupts the progress of CC

Cyclins

- proteins that form complexes with CDK, activate Cdk and determine their substrate specificity
- regulate the passage of the phases of CC
- its concentration fluctuates during the CC phases - specific synthesis / ubiquitination and proteolysis





Cyclins of G1-phase (D): Participation in the regulation of cyclins G1 / S

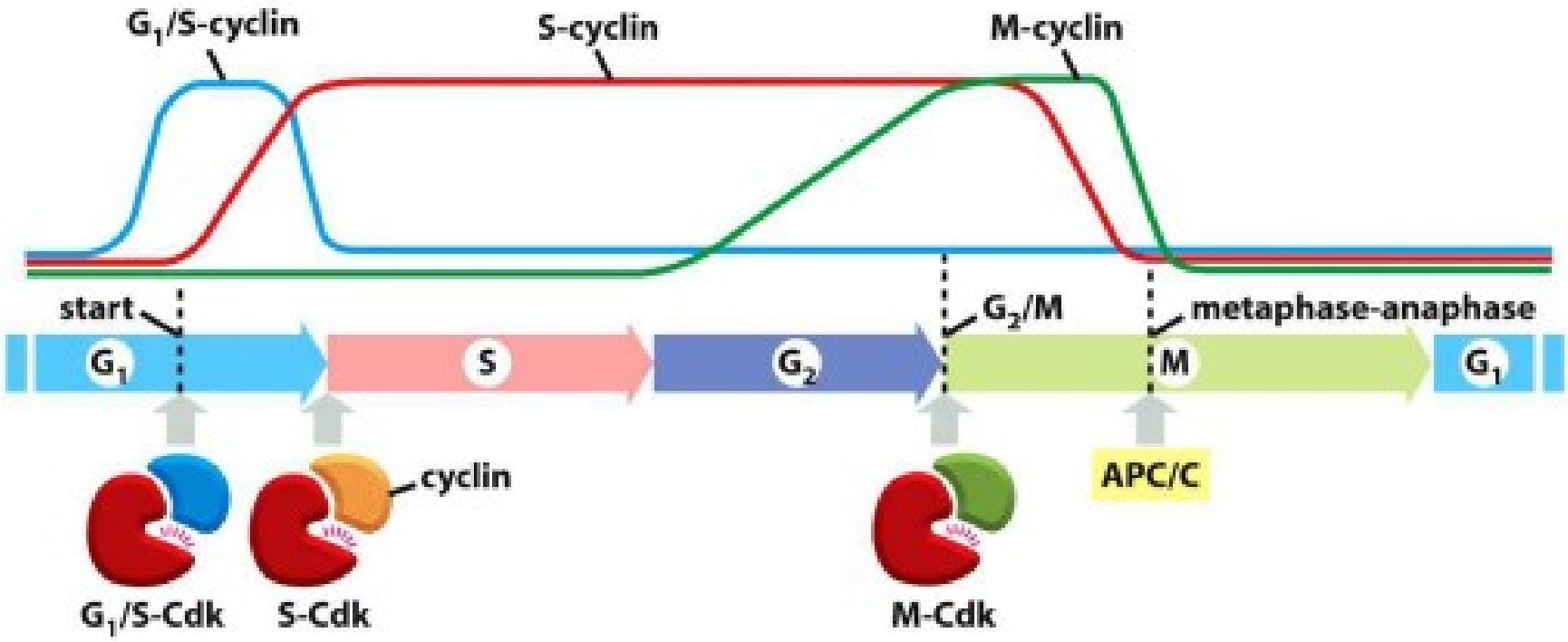
Cyclins of G1/S-phase (E): CDK activation in late G1, contributing to overcome the restriction point

Cyclins of S-phase (A): CDK activation point after the passage of restriction poinz, on duplication of chromosomes, the S phase cyclin remains elevated until mitosis, because they contribute to the regulation of early mitosis.

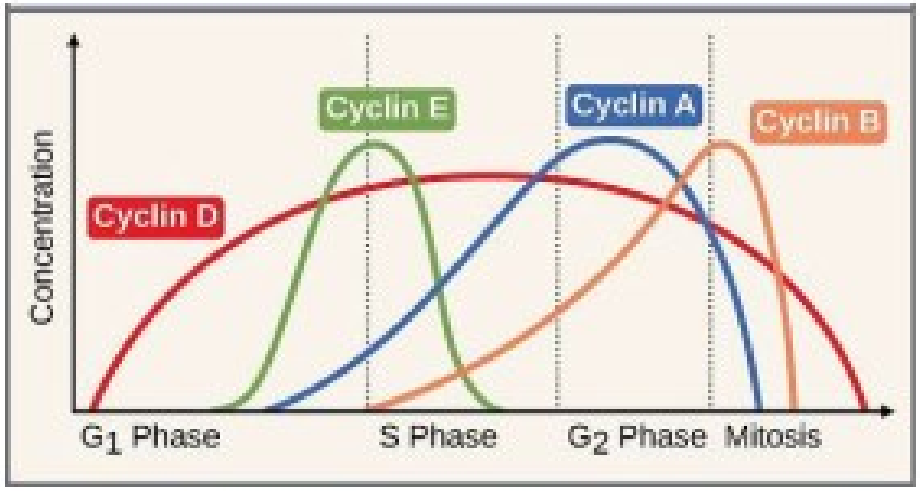
Cyclins of M-phase (B): Cdk activation stimulating entry into mitosis after passing through the **checkpoint G2 / M**, degradation in the middle of phase M.

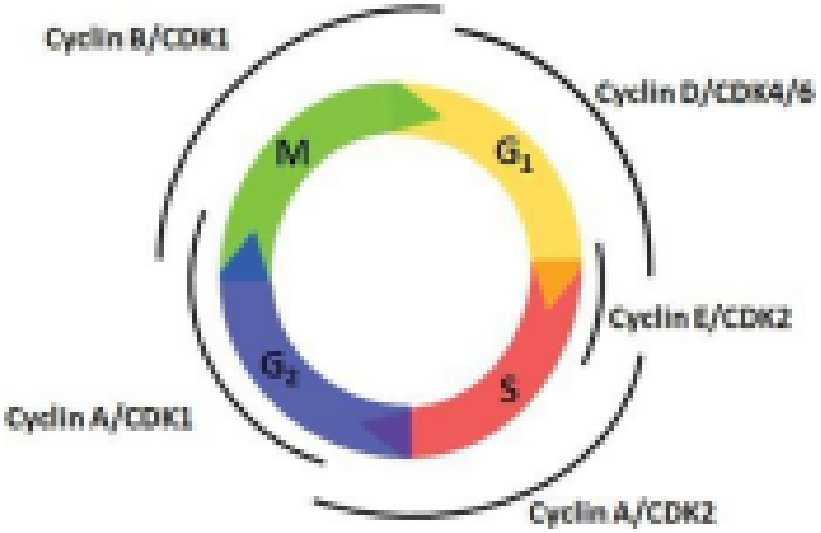
The activity of cyclin-cdk complexes

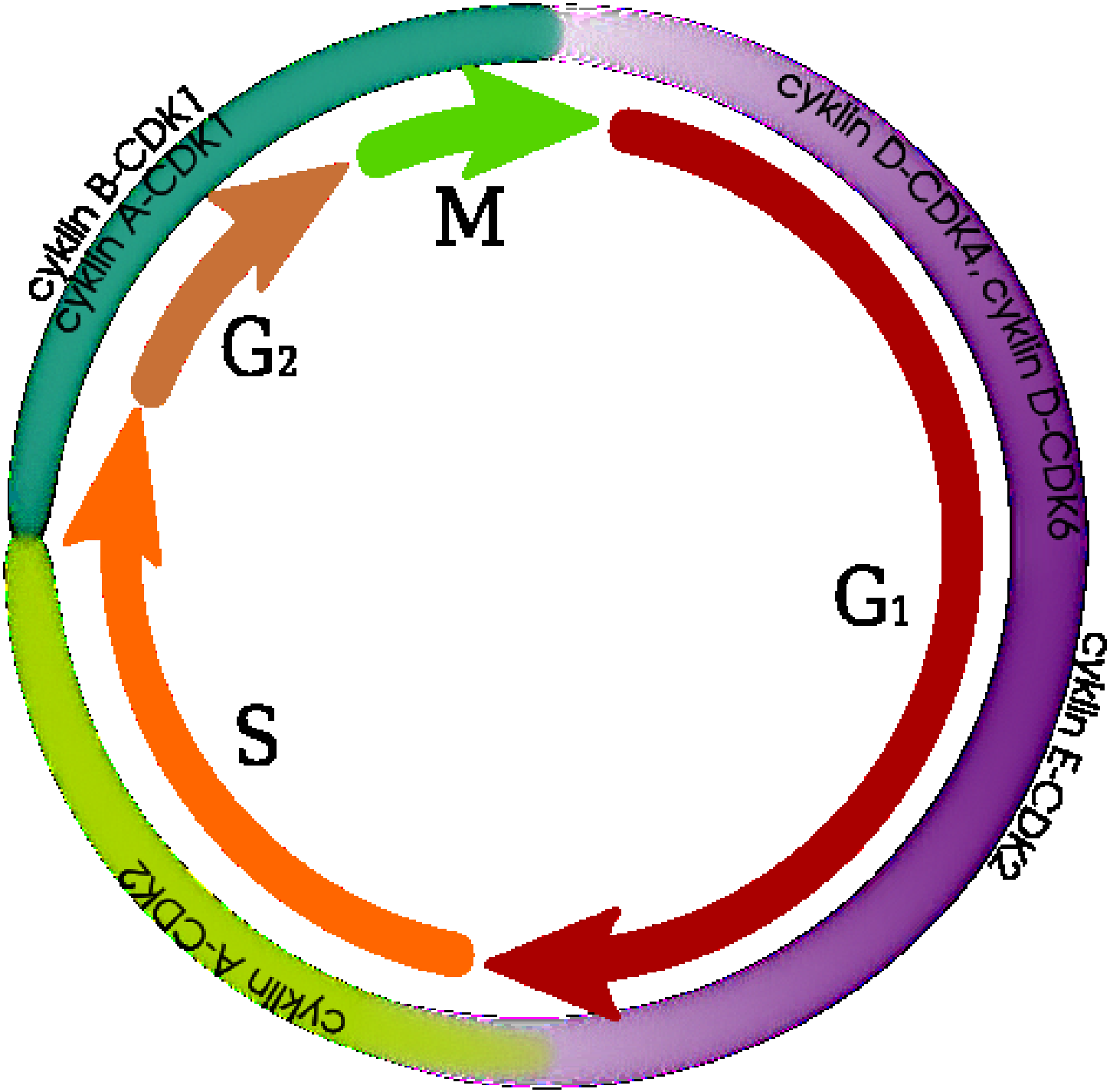
- concentration of cyclins varies during the cell cycle
- concentration of CDK is more stable, but their activity is zero without cyclin
- cyclins co-decide on the choice of substrate phosphorylation by complex CDK / cyclin

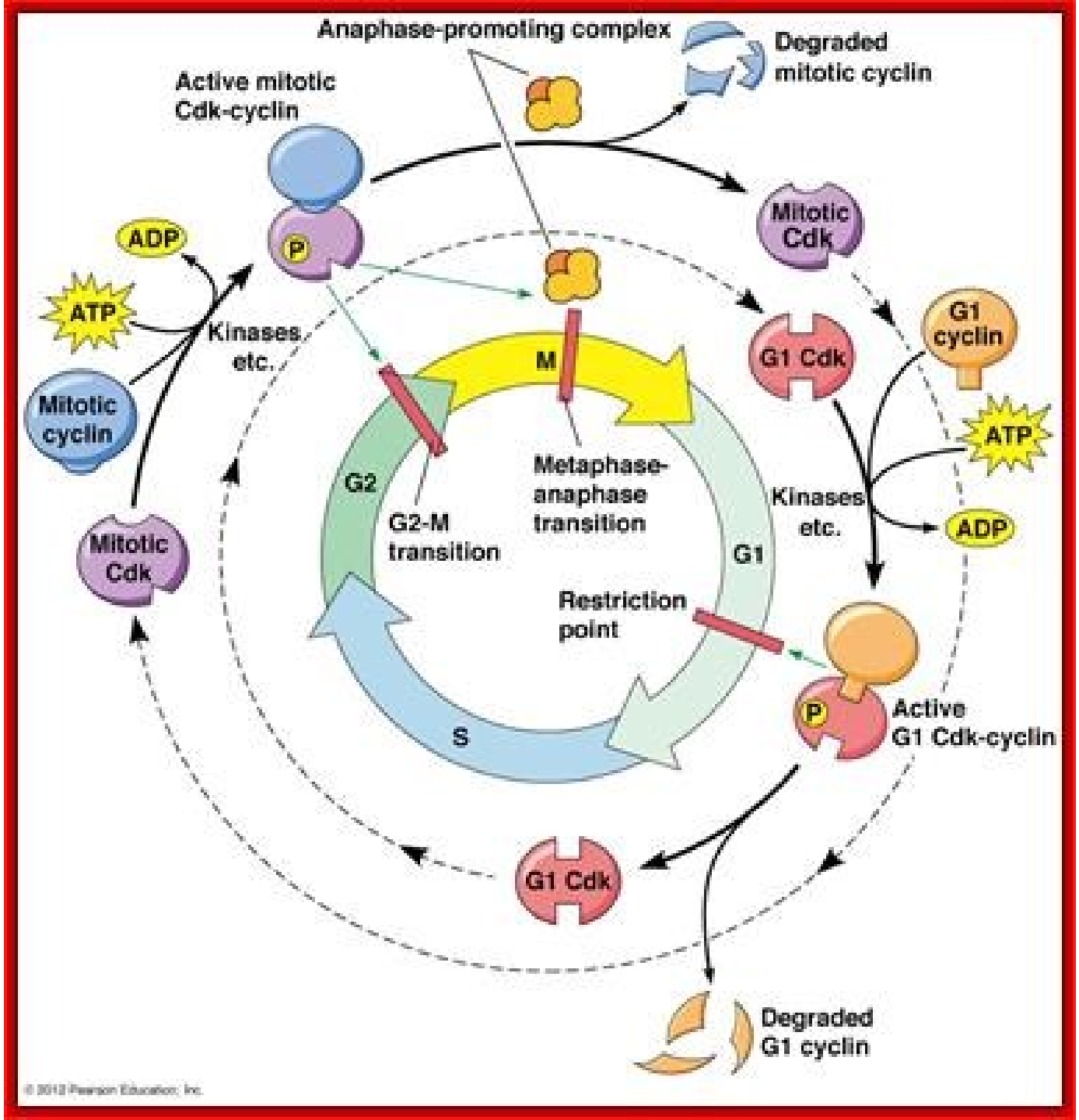


Fáze BC	Cyklin	CDK	Funkce
G1	D, E	CDK4, CDK6, CDK2	Umožňují překonat bod restrikce v pozdní G1
S	A, E	CDK2	Umožňují zahájit replikaci DNA
G2	A	CDK2, CDK1	Navádějí buňku k M fázi
M	B	CDK1	Podporují mitotické děje



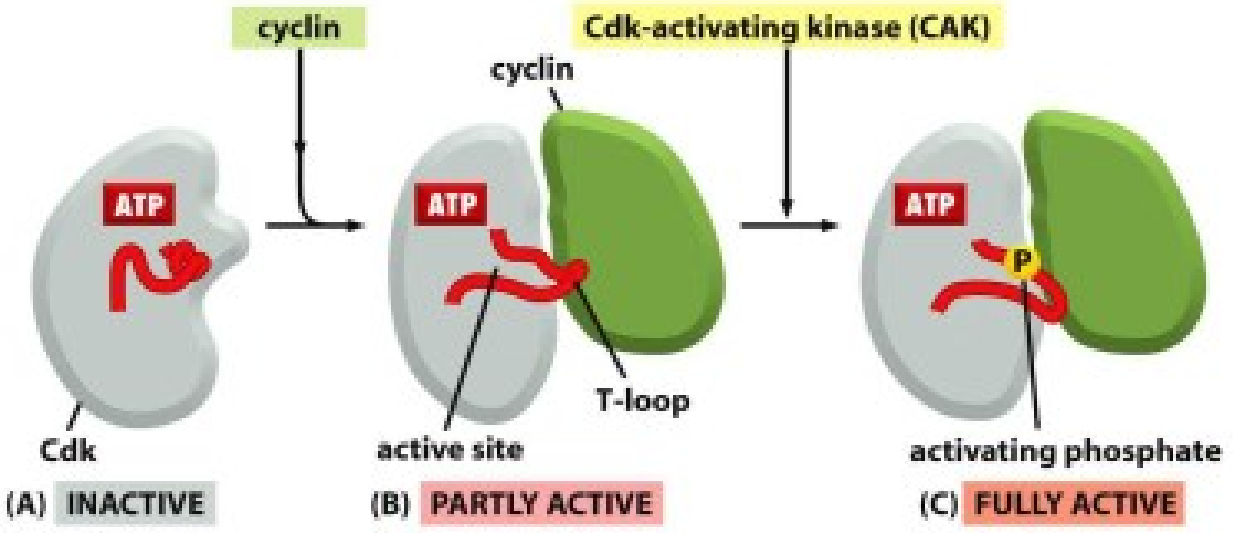



cyklin B-CDK1
cyklin A-CDK1
M
G2
S
cyklin A-CDK2
cyklin D-CDK4, cyklin D-CDK6
G1
cyklin E-CDK2


Anaphase-promoting complex
Degraded mitotic cyclin
Active mitotic Cdk-cyclin
Mitotic Cdk
ADP
P
ATP
Kinases etc.
Mitotic cyclin
M
G1 Cdk
G1 cyclin
ATP
G2
G2-M transition
Metaphase-anaphase transition
Kinases etc.
ADP
G1
Mitotic Cdk
Restriction point
P
Active G1 Cdk-cyclin
S
G1 Cdk
Degraded G1 cyclin
© 2012 Pearson Education, Inc.

Three functional states of Cdk

A) **inactive** - no cyclin - Cdk active site is blocked by the portion of the protein, called T-loop
(B) **partially active** - after binding of cyclin T-loop leaves the active site of Cdk and partially activates it
(C) **fully active** - threonine phosphorylation of the T-loop Cdk kinase by CAK (cdk-activating kinase): conformational changes, increased affinity for the respective substrates

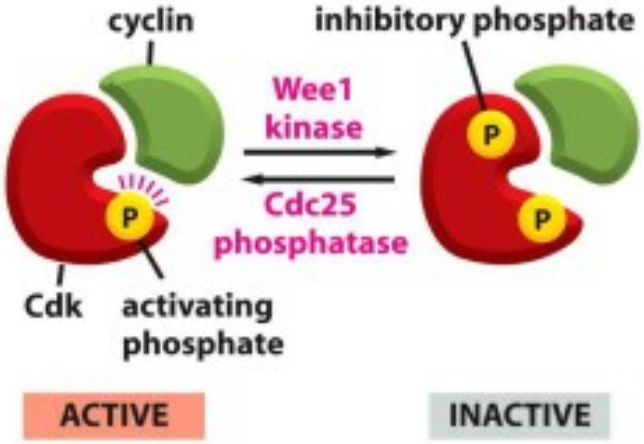


The regulation of CDK activity: a) phosphorylation

binding of cyclins primarily determines the activity of Cdk

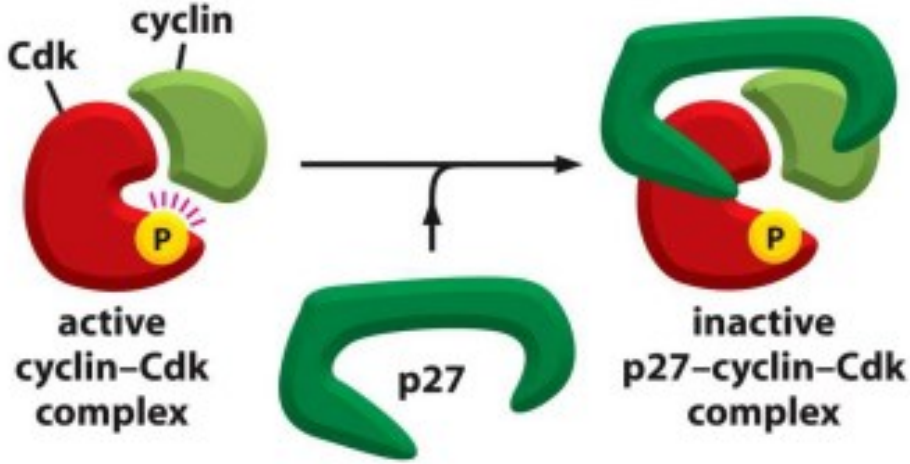
assistance mechanisms contribute to the fine regulation of CDK activity:

- phosphorylation of pair of amino acids near the active site of the kinase **Wee1 inhibits the activity of cyclin / CDK complexes**
- dephosphorylation of amino acids by phosphatase **Cdc25 enhancing activity of cyclin / CDK complexes**
- other activating phosphate adds CAK to T-loop

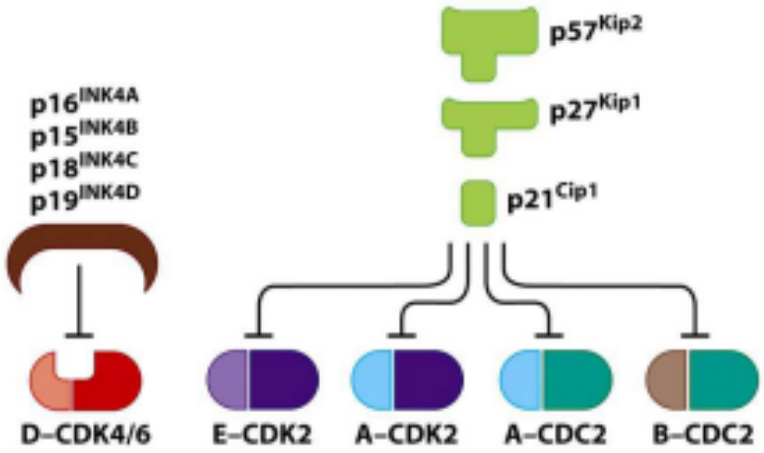


The regulation of CDK activity: b) inhibitory proteins CKIs

- bind to cyclin / CDK complexes and inhibit in
- due to changes in conformation of the active site in Cdk



a) family Cip/Kip
b) family INK4



CDK inhibitors: Cip/Kip family

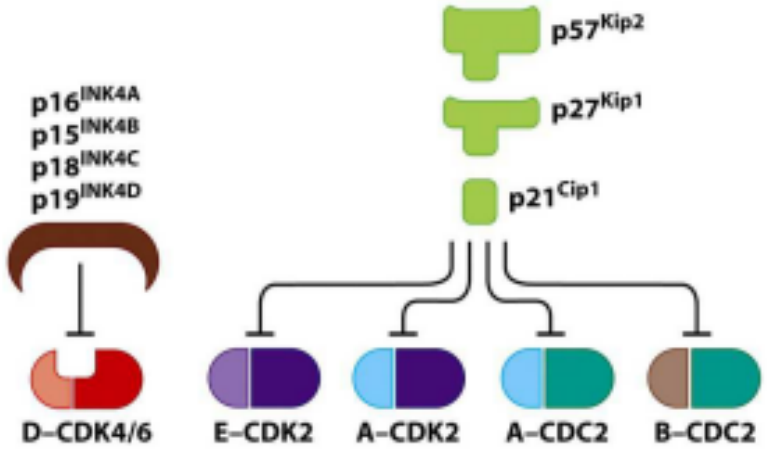
three members in mammals: p21 Cip1 , p27 Kip1 , p57 Kip2

distinct roles in cell cycle regulation

p21 is induced by DNA damage using p53 - ensures arrest of cell cycle in G1 and G2 phases

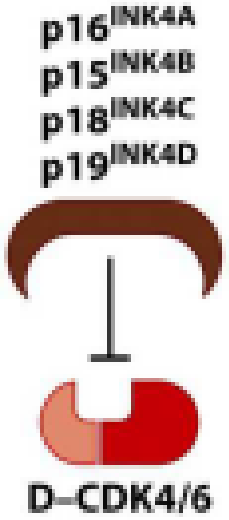
p27 applies when leaving the cell cycle when they accumulate; upon re-entry into the cell cycle from dormancy is rapidly degraded

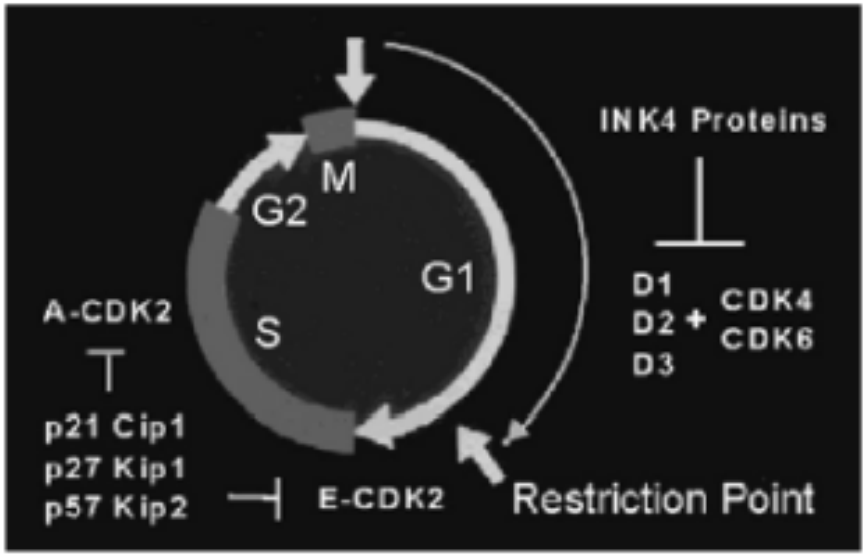
p57 is applied during embryogenesis, is expressed tissue-specifically during development



CDK inhibitors: INK4 family ("inhibitors of CDK4")

p16 INK4A
p15 INK4B
p18 INK4C
p19 INK4D



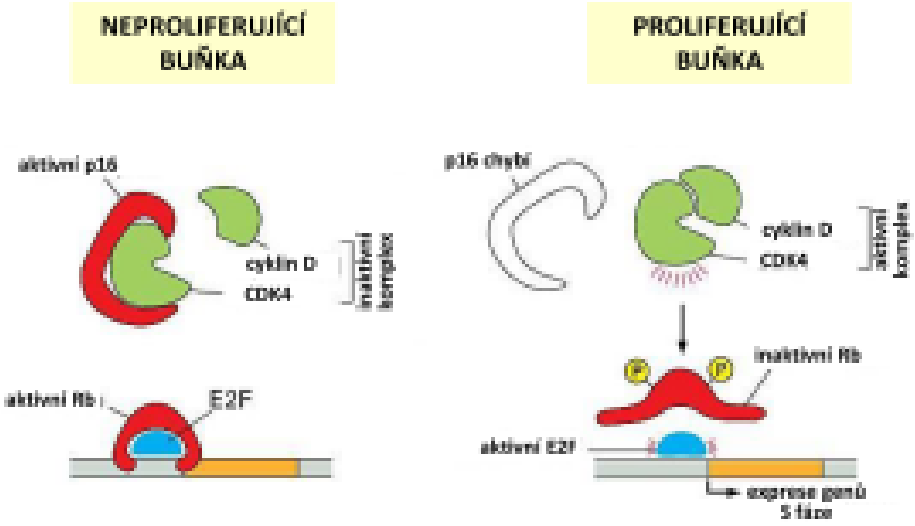


Common signs:

inhibiting the formation of a complex of cyclin D and CDK4 / CDK6 and inactivation of already formed complexes

cell cycle arrest in the **G1 phase (prior to the restriction point)**

• expression of p16 stimulated by growth inhibitors (e.g. TGFβ) and E2F (negative feedback)



Pharmacological inhibitors of CDKs

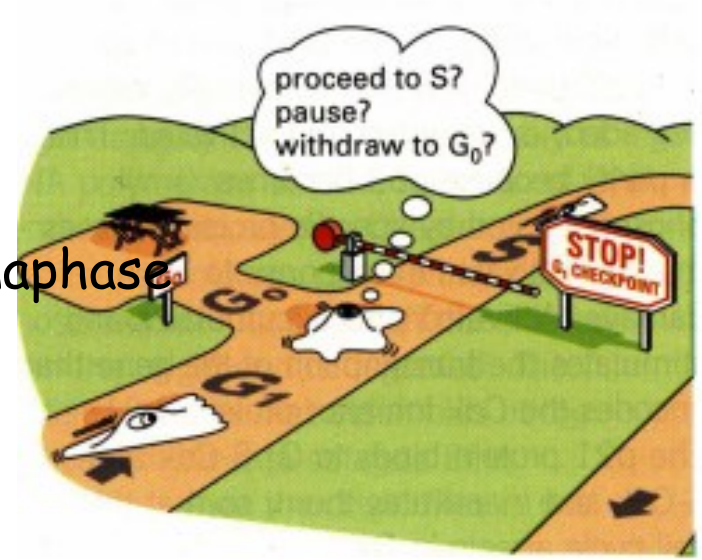
- CDKs contol cell cycle, control gene expression, differentiation, apoptosis, processes in the nervous system
- deregulation of CDKs in diseases, **pharmacological inhibitors blocking the ATP binding site of CDK**
- investigation of the selective action of these inhibitors and their possible use for medical purposes
- - tumors, neurodegenerative diseases (Alzheimer's disease, ALS, stroke)
- - cardiovascular diseases (atherosclerosis, heart hypertrophy)
- - viral infections (HCMV, HIV, HSV, HPV)
- - parasitic protozoa (malaria, sleeping sickness)

Palbociclib (PD-0332991) (inhibitor of CDK4 and CDK6) gave encouraging results in a phase II clinical trial on patients with estrogen-positive, HER2-negative advanced breast cancer

CC checkpoints-molecular brakes

They control :

- ✓ initiate S phase
- ✓ initiation of mitosis
- ✓ distribution daughter chromosomes in anaphase
- ✓ beginning of telophase and cytokinesis
- ✓ DNA integrity



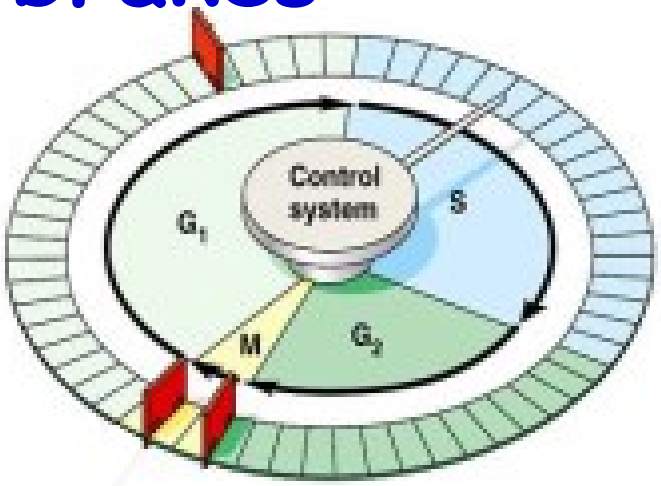
ensure that major events of cell cycle tate place in a fixed order

sensors monitor key going cycle and provide a feedback control system

if the error is logged, the control system will extend the stage that the error could be corrected

provide extremely accurate cell division (receiving the correct number of chromosomes correctly replicated by daughter cells)

CC checkpoints-molecular brakes

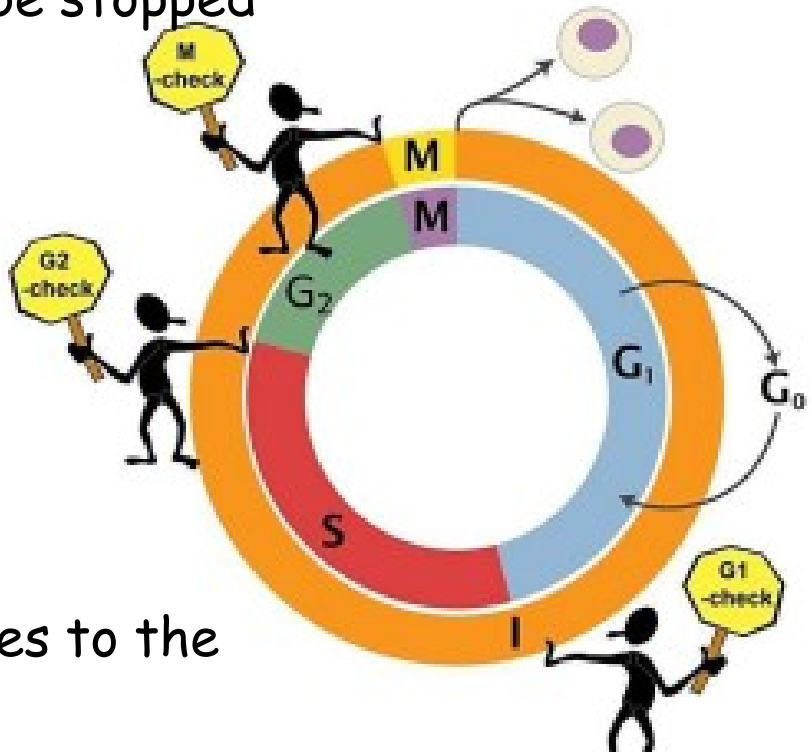


They perceive external and internal stimuli and ensure the proper operation of CC
• checking the completion of the previous phase, and fulfillment of conditions for the next phase

G1 checkpoint - restriction point
• external factors - the presence of nutrients, mitogens, antiproliferative factors
• after crossing restriction ponit, CC can be stopped only by internal factors
• DNA integrity
• size of cells (yeast)

G2 checkpoint
• completion of DNA replication
• DNA integrity
• size of cells (yeast)

M checkpoint
• metaphase-anaphase transition
• proper connection of mitotic chromosomes to the spindle



External stimuli - chemical

• animal cells require addition of essential nutrients, extracellular signals for growth, division and survival

• **signaling molecules produced by other cells of the same organism:**

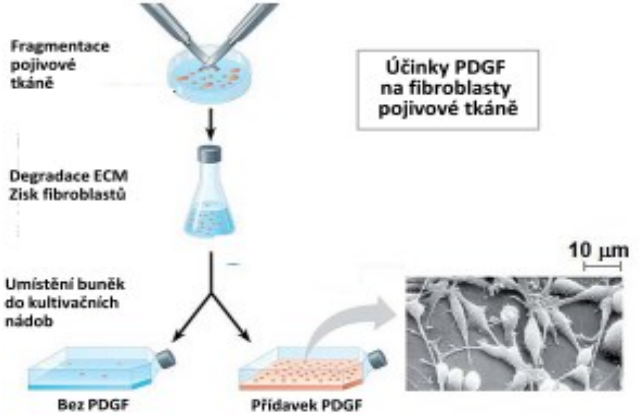
i) mitogens - stimulate cell division, overcome natural braking mechanisms G1 / S (e.g. Rb)

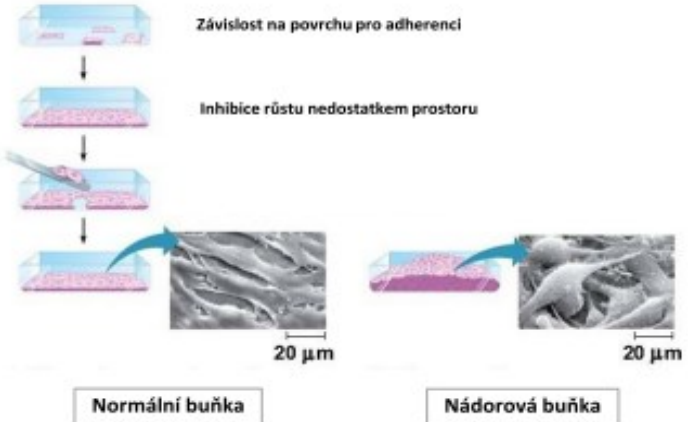
ii) growth factors - stimulate increase in mass and cell growth, induce the synthesis of macromolecules - e.g. PDGF: formation in platelets, promotion of wound healing

iii) factors for survival - suppress programmed cell death

External stimuli – physical

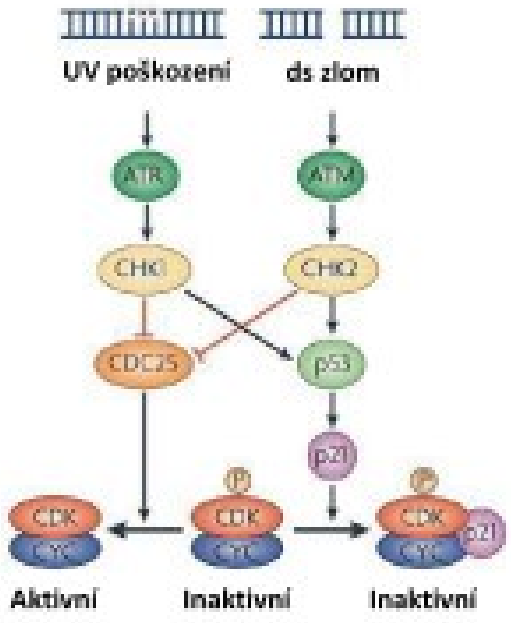
for growth and proliferation cells require space and surface for adherence

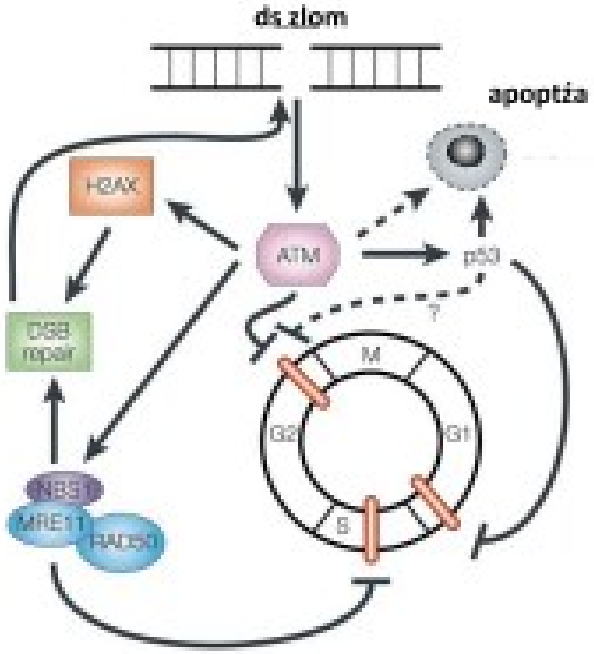




Internal stimuli • DNA integrity, connecting chromosomes to the spindle

DNA damage





due to physical and chemical mutagens

- **repairable damage – stop of CC to the time of repairing DNA**
- **extensive damage – programmed cell death**
- **CC arrest in G1 (inactivation of CDK2) or G2 (Cdk1 inactivation)**
- the key role of tumor suppressor genes

  ATM, ATR - recognition of DNA damage (UV photoproducts, dsDNA breaks), activation of CHK1 CHK2

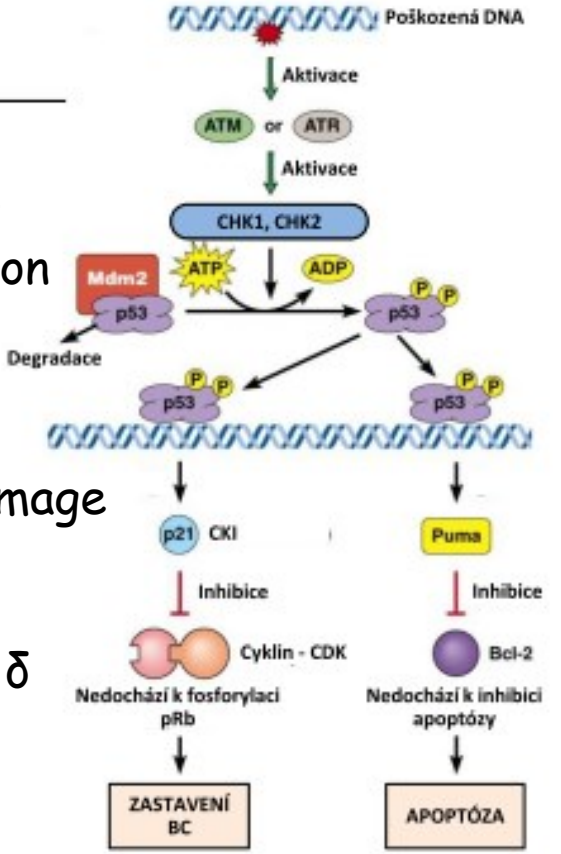
  CHK1, CHK2 – activation of p53, inhibition of Cdc25

  p53 - increased levels of CKIs

Cell cycle checkpoints

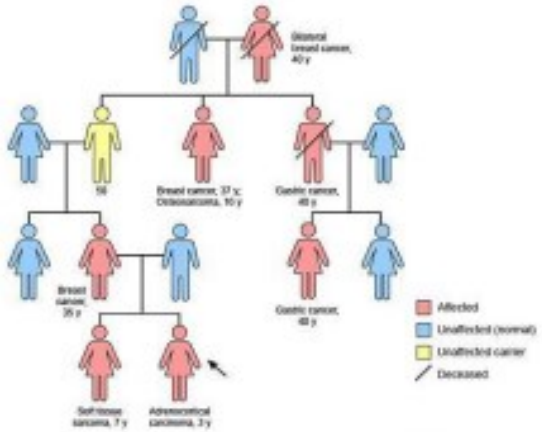
p53

- important cell cycle regulator, acts as a tumor suppressor
- Under normal conditions, a low level, continuous degradation
  - Mdm2 provides transport p53 from the core to the proteasome
  - Feedback: p53 triggers the expression of Mdm2
- stabilized in stressful situations, especially when DNA damage
- acts as a transcription factor, induces
  - cell cycle arrest: expression of p21/CIP
  - stop DNA replication: inactivation of DNA polymerase δ
  - mitosis arrest: inactivates cdc25
  - apoptosis: expression of Bax, Puma



p53 deficiency

- through the cell cycle with damaged DNA - the accumulation of mutations – risk of tumors (mutations in the p53 gene in the majority of cancer)
- Li-Fraumeniho syndrome
  - hereditary syndrome caused by p53 inactivating mutations
  - increased risk of cancer (sarcomas, brain tumors, breast cancer, adrenal gland, leukemia)
  - 50 % risk of cancer up to 40 years of age, 90% to 60 years of age



Cell cycle checkpoints

Spindle checkpoint

• for entry into anaphase cells must receive confirmation of the connection of all chromosomes to spindle microtubules

• damage to this control mechanism leads to damage and nondisjunction of chromosomes

i) checkpoint ON

• unattached kinetochore activate Mad and Bub proteins

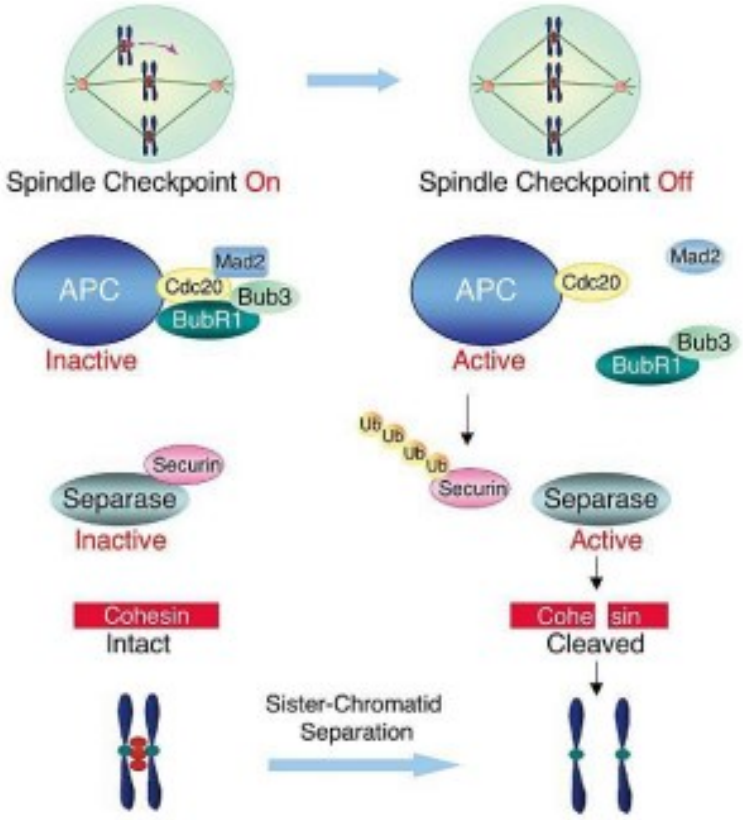
• complex APC-Cdc20 in inactive state

ii) checkpoint OFF

• Sister chromatid chromosomes binding on microtubules from opposite ends of spindle causes mechanical stress in the chromosomes

• complex of proteins Mad and Bub not formed

• APC-Cdc20 complex is active and provides securine degradation



Mitogenes

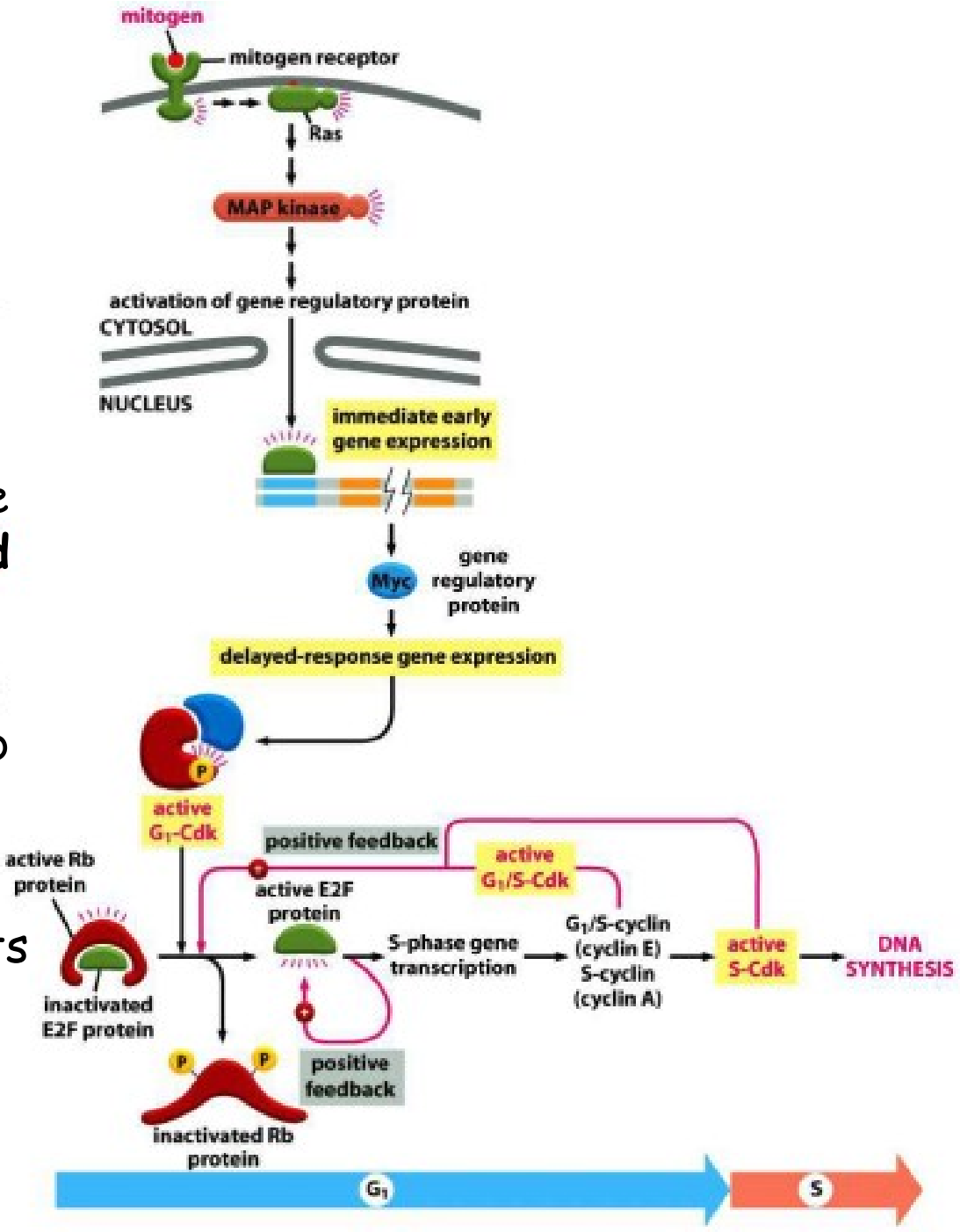
pro-growth signaling molecules, that activate **genes of immediate response** by intracellular signaling cascades

products of immediate response genes activate genes of **delayed reaction (eg. cyclin D)**

cyclin D/CDK phosphorylates Rb

positive feedback: **E2F**

enhances the transcription of its own gene; G1/S-Cdk and S-Cdk increased phosphorylation Rb



E2F proteins

E2F are transcription factors that activate gene expression encoding proteins necessary for DNA replication, cyclins of late G1 and S phase and CDK of S phase

The absence of mitogenes:

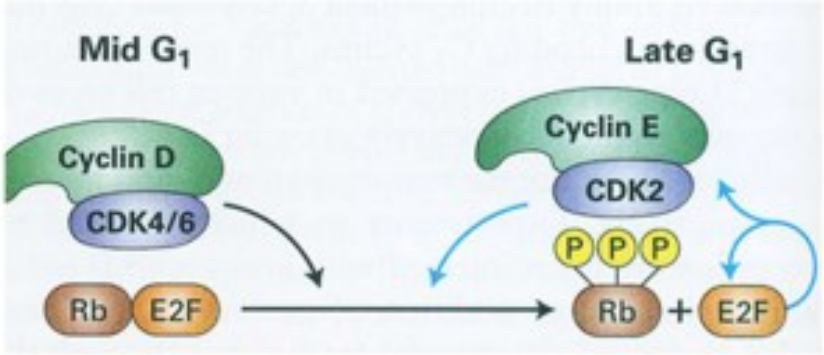
gene expression controlled by E2F is unabled by interaction between E2F and Rb protein

The presence of mitogens:

G1-CDK phosphorylates Rb,

Rb-E2F bound is released

and E2F subsequently ensure expression of target genes



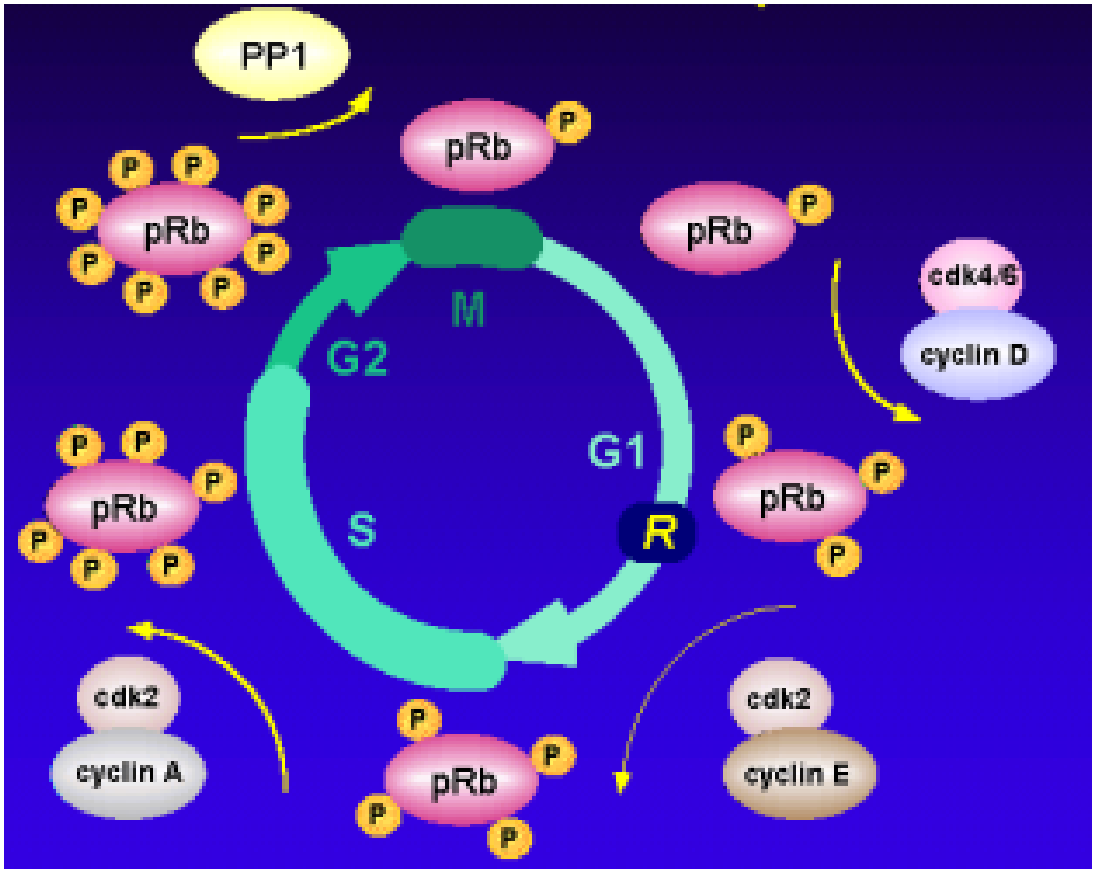
Crossing point restrictions: participation of Rb

Rb protein is a key substrate of cyclin-CDK complex of G1 phase

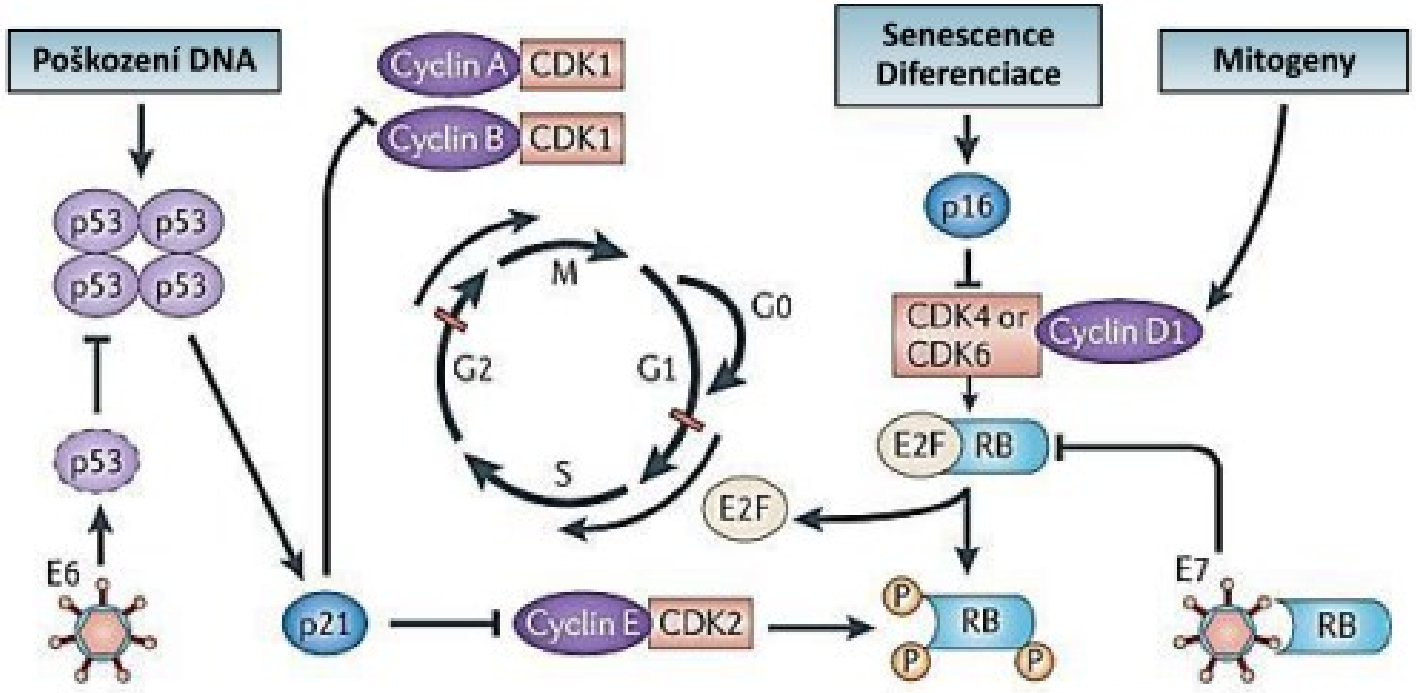
phosphorylation Rb in several places prevents its association with E2F

phosphorylation Rb is further increased during a cell cycle

by phosphatase PP1 Rb passes to the hypophosphorylated state of G2 phase



Factors influencing the passage of the cell cycle



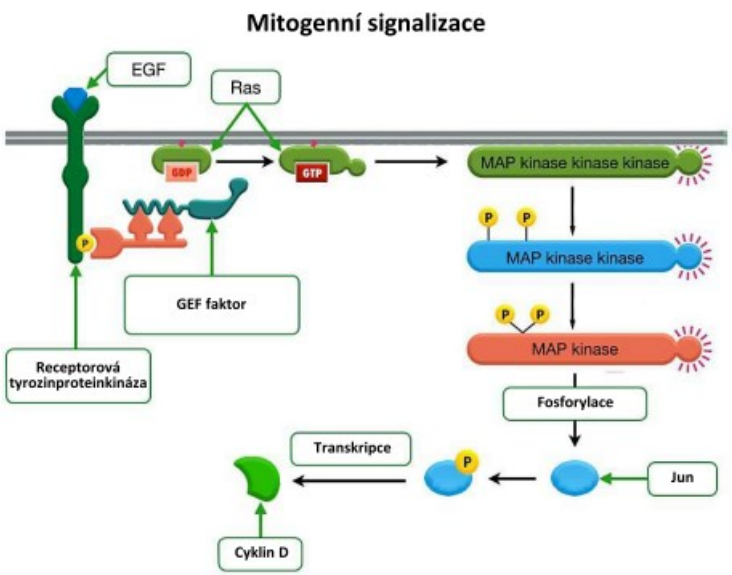
presence of mitogenes – expression of cyclin D

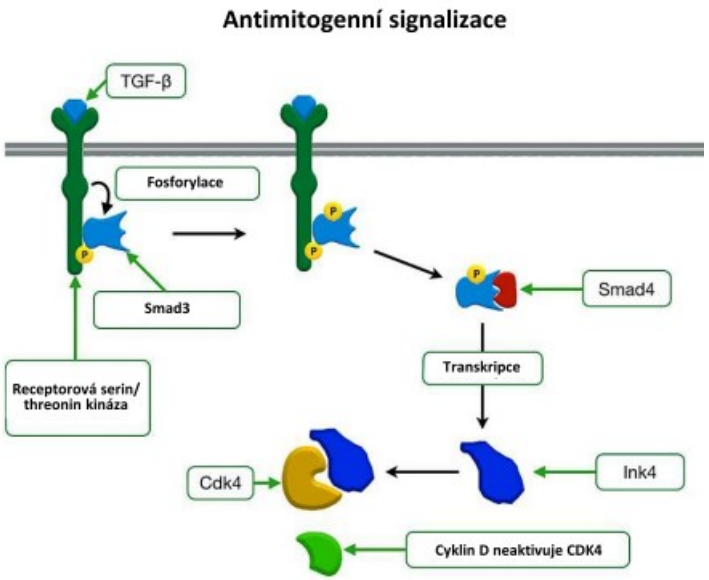
- presence of antimitogene, differentiation factors – expression of CKI
- cell aging – expression of CKI
- damage of DNA – arrest of cell cycle through p53
- viral infections (eg. HPV, human papillomavirus) – inhibition of p53 and pRb, cell proliferation

Cell cycle control – early phase of G1

- **cyclin D – CDK4/6**
- Three forms of cyclin D (D1, D2, D3) with tissue-specific expression form complex with CDK4 or CDK6
- cyclin D acts as a sensor of mitogenic signals and prepares the cell for the S-phase
- activation of transcription factors, which provide expression of replication proteins and cyclin E
- dephosphorylation of components pre-initiation complexes, building of new complexes in places ori
- **response to mitogenic signals:** eg. EGF - EGFR - GEF factor - Ras protein - MAP kinase pathway - transcription factors – expression of cyclin D
- **inhibition of antimitogenes signals**

  eg. TGFβ - TGFβR - transcription factor Smad3, Smad4 – expression of CDK inhibitor

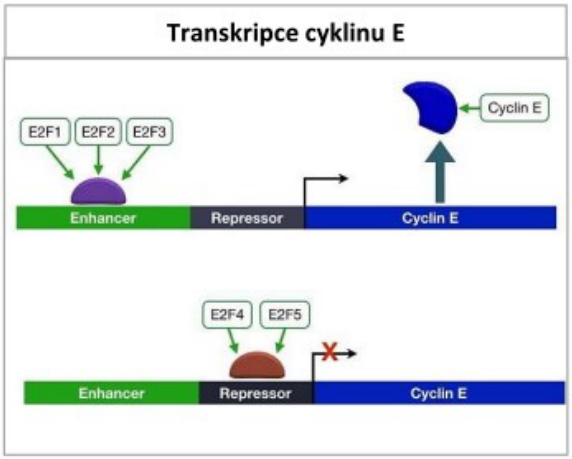


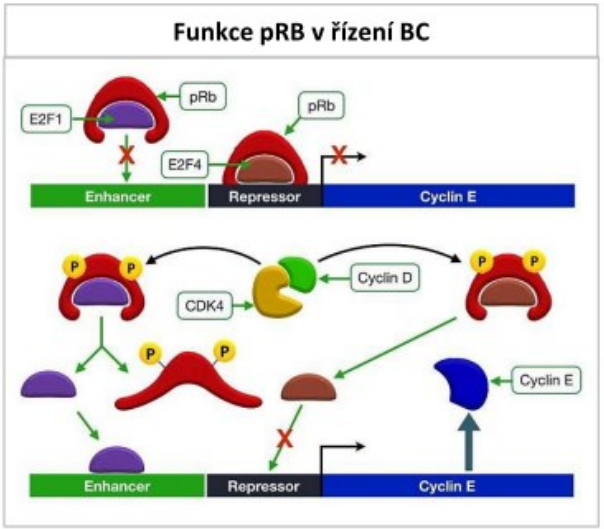


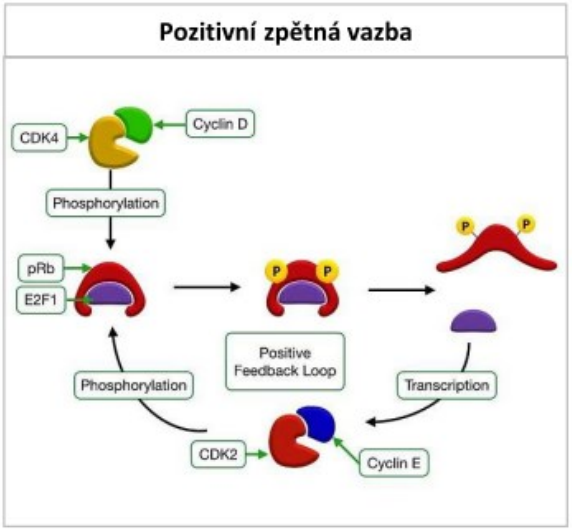
Cell cycle control – transition of G1/S

cyclin D - CDK4/6, cyclin E - CDK2

- further prepare the cell to the S phase
- transcription of cyclin E controlled by a set of transcription factors E2F (activators 1-3, inhibitors 4-5)
- Rb protein (pRb) active in unphosphorylated form, inhibited by phosphorylation
- in G0 and G1 pRB inhibits by its binding activating E2F and activates E2F inhibition, suppresses the expression of cyclin E
- after phosphorylation by complex cyclin D - CDK4/6 pRb is released and E2F triggers expression of cyclin E, cyclin A and replication proteins
- Positive feedback – cyclin E formes complex with CDK2 and complets pRb phosphorylation, at this point it is through the cell cycle independent of the presence of a mitogen
- after initiation of S phase, cyclin E is degraded and CDK2 forms a complex with cyclin A







Cell cycle control – S phase

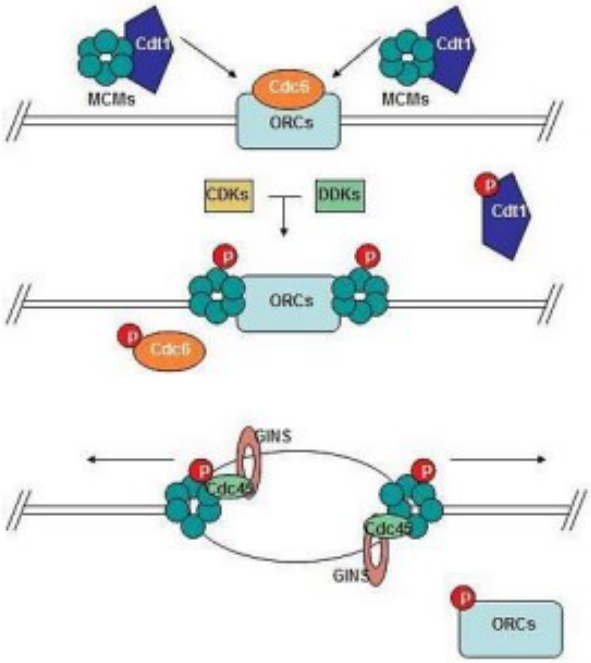
cyclin A - CDK2

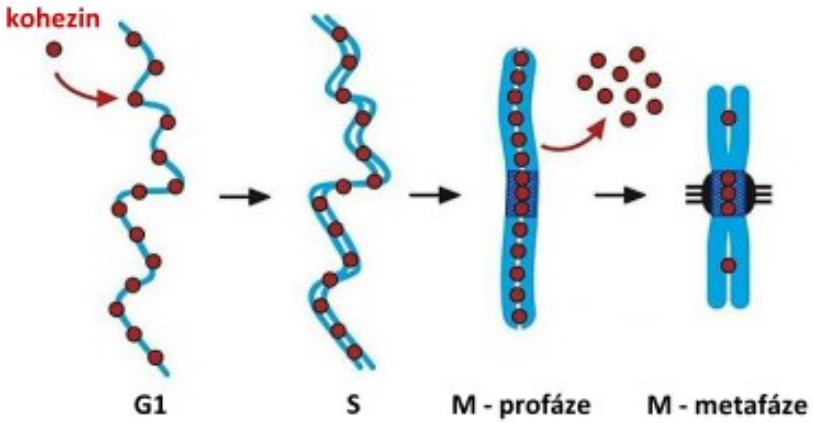
• phosphorylation of components of the pre-initiation complex

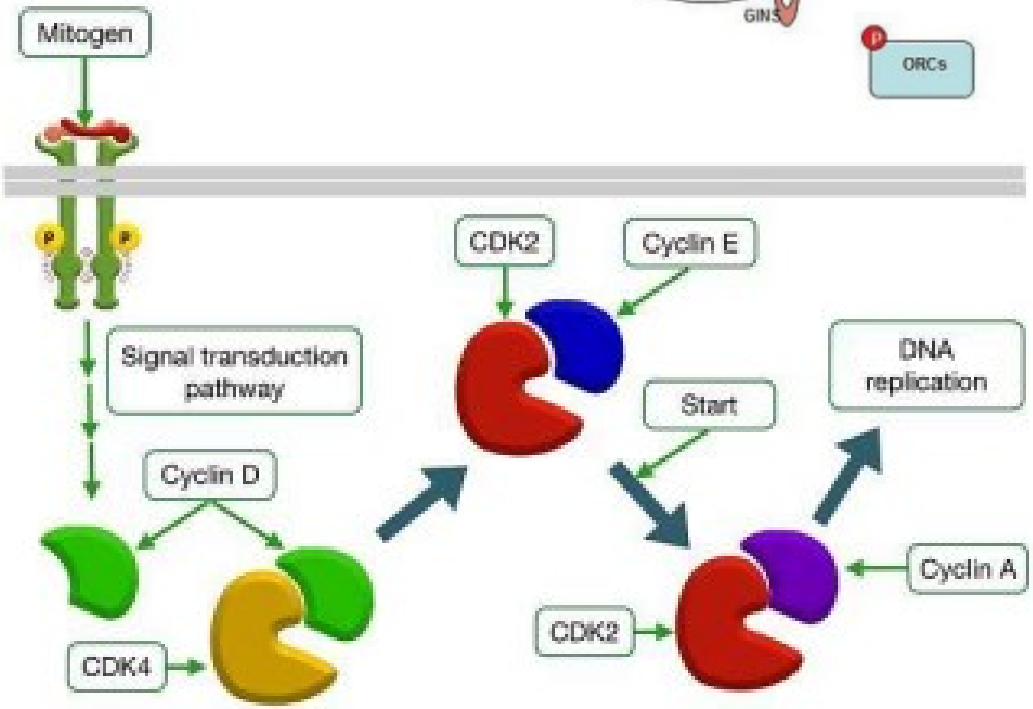
- initiation of DNA replication (activation complex MCM)

- block of repeat replication within a cell cycle (cdc6 releasing)

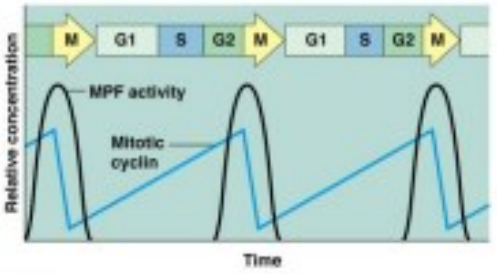
• The result of S phase is the emergence of two sister chromatids of each chromosome associated by cohesin molecules





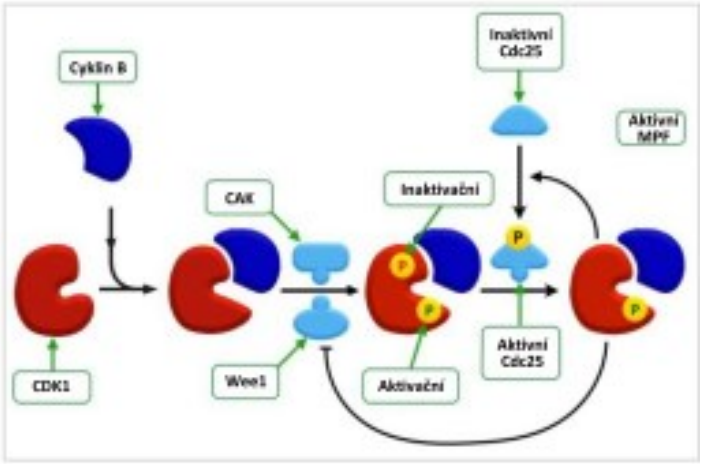


Cell cycle control – transition of G2/M



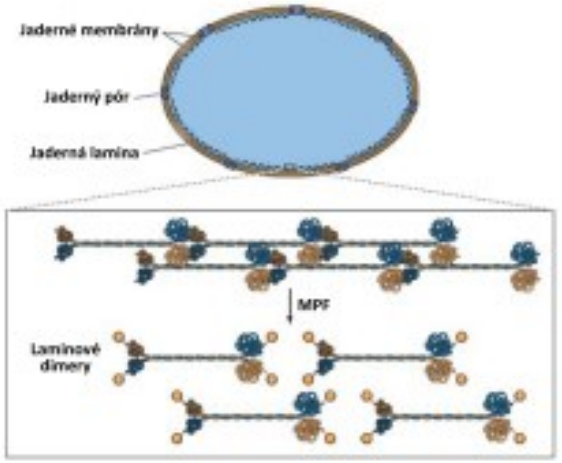
MPF (mitosis-promoting factor) = cyclin B - CDK1

- cyclin B concentration increases continuously throughout interphase
- CDK1 - inhibitory kinase phosphorylation by Wee1
  removed by phosphatase Cdc25
  - activating phosphorylation by kinase CAK
- increase in MPF activity is jumping, at maximum level of cyclin B and after removal of inhibitory phosphorylation
- Positive feedback - activated MPF activates Cdc25 and inhibits Wee1



MPF phosphorylates target proteins involved in early stage of mitosis

- chromatin condensation - activation of condensins
- disintegration of the nuclear membrane – depolymerization of laminates, phosphorylation of nuclear pore and proteins of inner membrane
- formation of the mitotic spindle - activation of centrosomes and proteins associating with microtubules



Control of cell size in yeast cells

- low activity of Cdc25 and Wee1 high activity - delayed mitosis, prolonged G2, cell enlargement
- high activity of Cdc25 and low activity of Wee1 - Fast Track mitosis, shorter G2, reduce cell

Cell cycle control- M phase

APC, anaphase-promoting complex

• ubiquitin ligase connecting ubiquitin to proteins that control mitosis, and thus predetermines degradation

• activity during the cell cycle fluctuates, activated in late mitosis by treatment MPF

• only works when connected Cdc20 protein, the protein is blocked by Mad and Bub, who released him up after connecting the kinetochore of chromosomes to spindle microtubules

• substrate are e.g.:

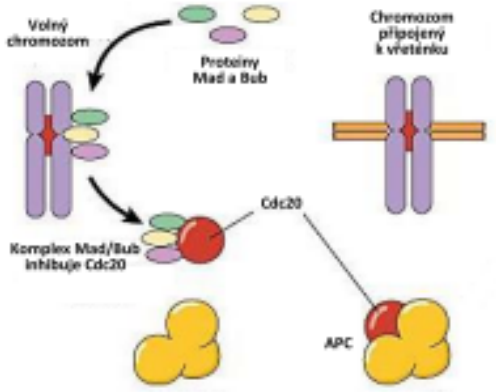
i) securin

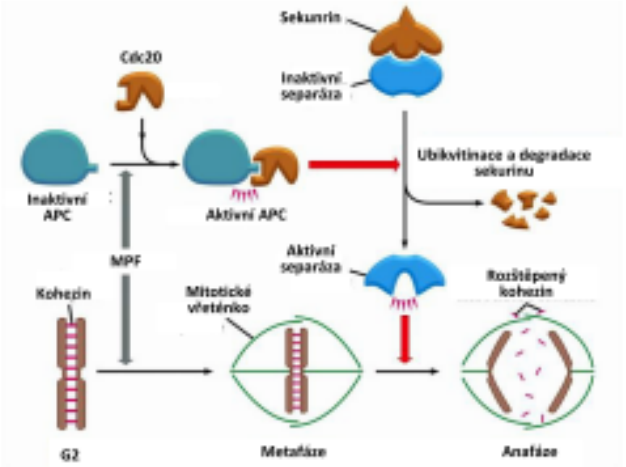
• its decomposition relaxes separase which degrades proteins linking sister chromatids

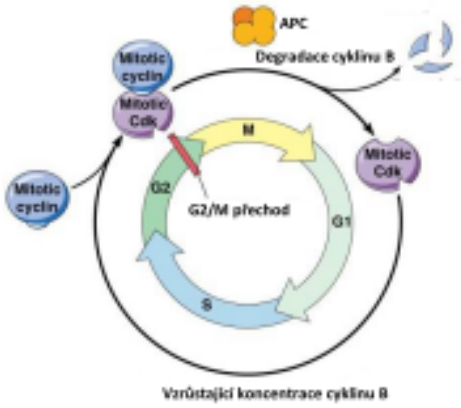
ii) cyclin B

• a sudden drop in his level inactivates MPF

• absence of MPF allows constitutively active phosphatases to remove phosphate groups from proteins that were phosphorylated MPF, mitotic endings, entry into a new G1



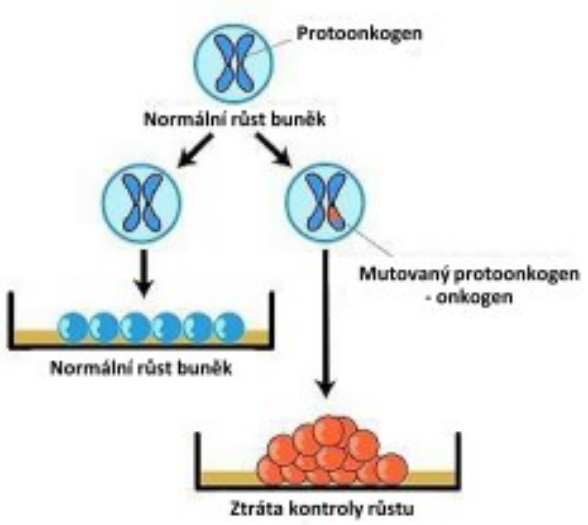




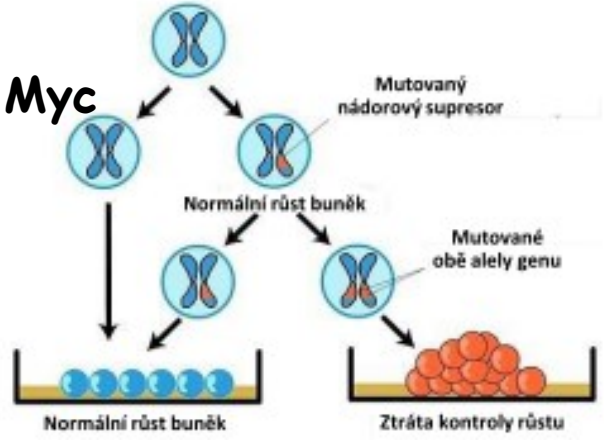
Disorders of cell cycle control

Tumour diseases

- tumor cells do not need to divide the growth factors
  - themselves can generate growth factors
  - May signal in the absence of growth factor
  - May have impaired cell cycle checkpoints
- increased proliferation of cells to support its transformation into **tumor cells**
- tumor is basically failure to control cell division, uncontrolled cell growth



- **protooncogenes**
  - normally activate cell proliferation
  - an abnormal activation or losing of inhibition - support tumorigenesis
- eg. **growth factors, cyclines, CDK, E2F, Mdm2, Ras, Myc**
- **tumor suppressors**
  - normally suppress cell division
  - inactivation or increased inhibition promotes tumor formation
  - **eg. pRb, p53, p21, p14, p16, TGF-β, ATM**



Disorders of cell cycle control

Numerical chromosomal abnormalities (aneuploidy)

• normal karyotype 46, XX(Y) monosomy - eg. 45, X trisomy - eg. 47, XY +21

• Errors in the mitotic spindle checkpoint, the result of delays and non-disjunction of chromosomes

i) Errors in mitosis

• chromosome delays in anaphase and non-inclusion in the core subsidiary

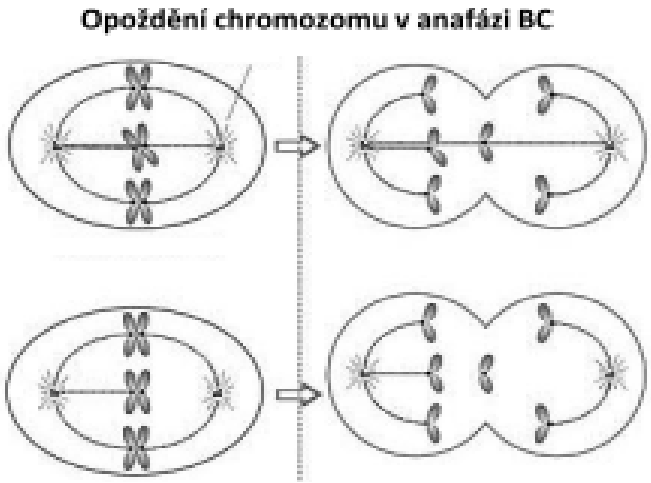
• formation of mosaicism, in one organism occurring cell lines with different karyotype

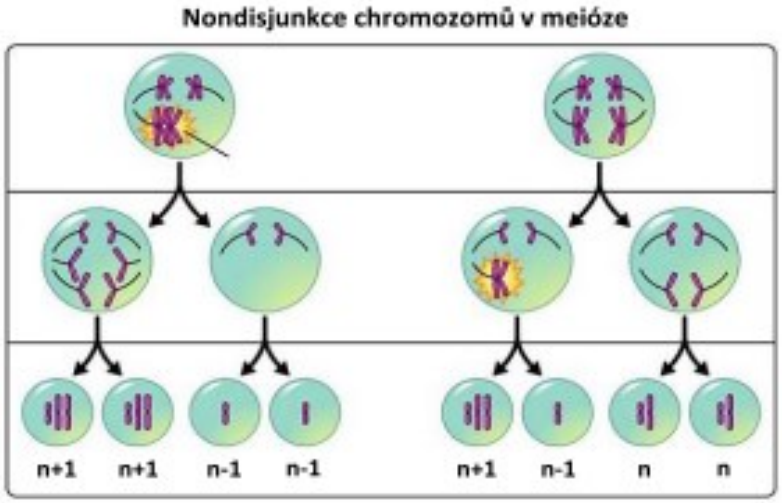
ii) Errors in meiosis

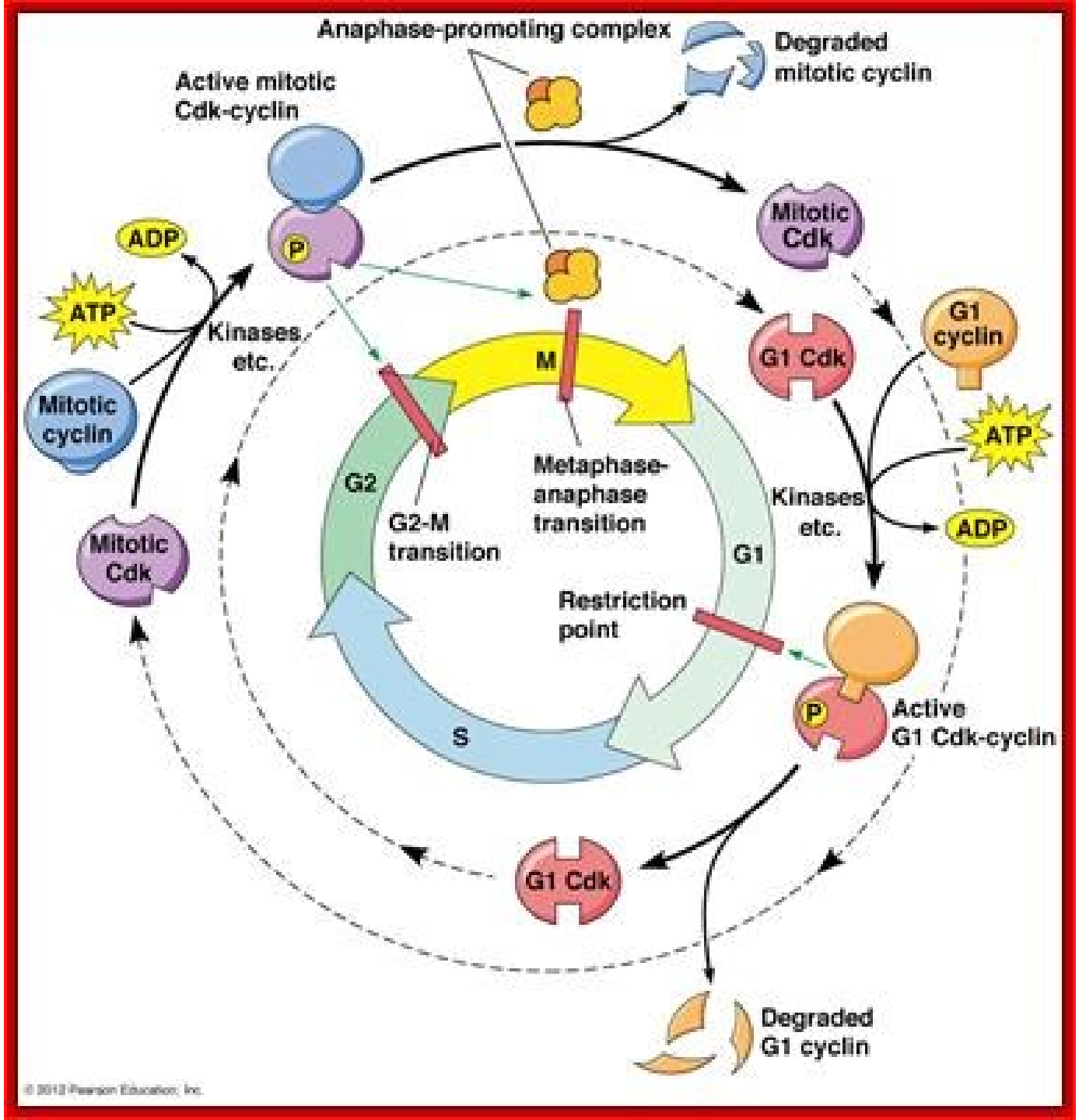
• non-disjunction - creation of nulisomic/trisomic gametes monosomy/trisomy after fertilization

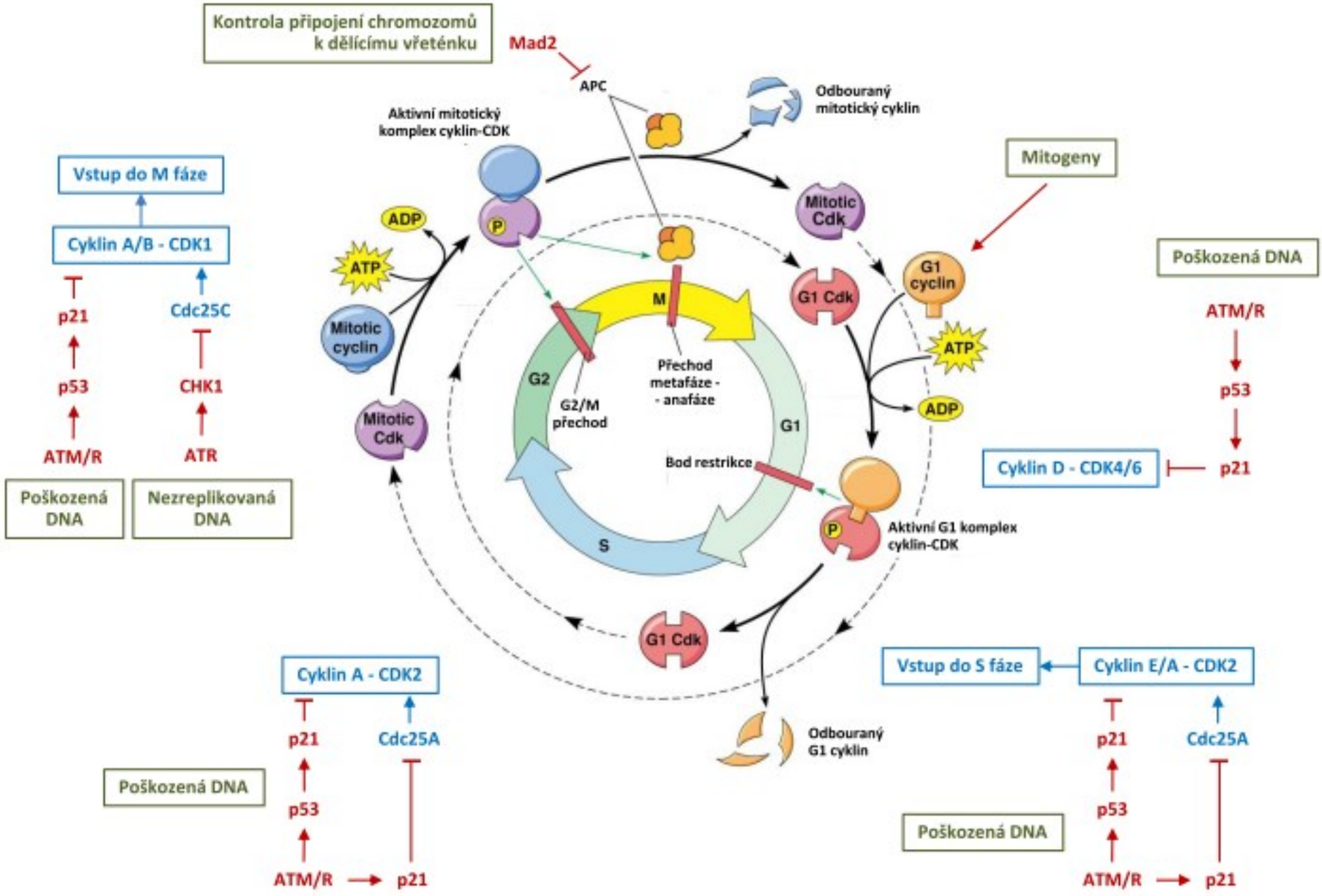
- in MI: error in the distribution of homologous chromosomes
- in MII: error in the chromatids division

• chromosome delays in anaphase : the formation of nulisomic gamete






Anaphase-promoting complex
Degraded mitotic cyclin
Active mitotic Cdk-cyclin
Mitotic Cdk
ADP
P
ATP
Kinases etc.
Mitotic cyclin
M
G1 Cdk
G1 cyclin
ATP
G2
G2-M transition
Metaphase-anaphase transition
Kinases etc.
ADP
G1
Mitotic Cdk
Restriction point
P
Active G1 Cdk-cyclin
S
G1 Cdk
Degraded G1 cyclin
© 2012 Pearson Education, Inc.


Kontrola připojení chromozomů k dělícímu vřeténku
Mad2
APC
Odbouraný mitotický cyklin
Aktivní mitotický komplex cyklin-CDK
Mitogeny
Vstup do M fáze
Cyklin A/B - CDK1
p21
Cdc25C
p53
CHK1
ATM/R
ATR
Poškozená DNA
Nezreplikovaná DNA
ADP
ATP
P
Mitotic Cdk
Mitotic cyclin
M
G1 Cdk
G1 cyclin
G2
G2/M přechod
Přechod metafáze - - anafáze
G1
Bod restrikce
S
Aktivní G1 komplex cyklin-CDK
Poškozená DNA
ATM/R
p53
p21
Cyklin D - CDK4/6
Cyklin A - CDK2
p21
Cdc25A
Poškozená DNA
p53
ATM/R
p21
Odbouraný G1 cyklin
Vstup do S fáze
Cyklin E/A - CDK2
p21
Cdc25A
Poškozená DNA
p53
ATM/R
p21