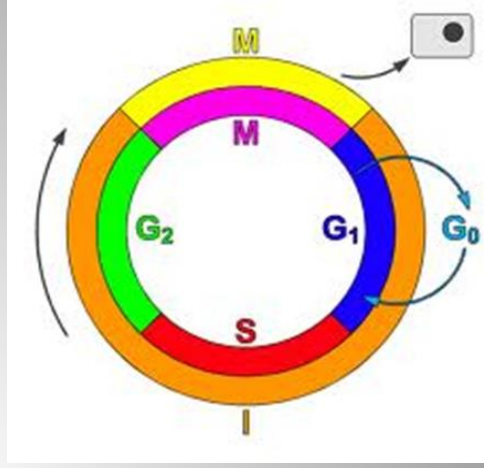
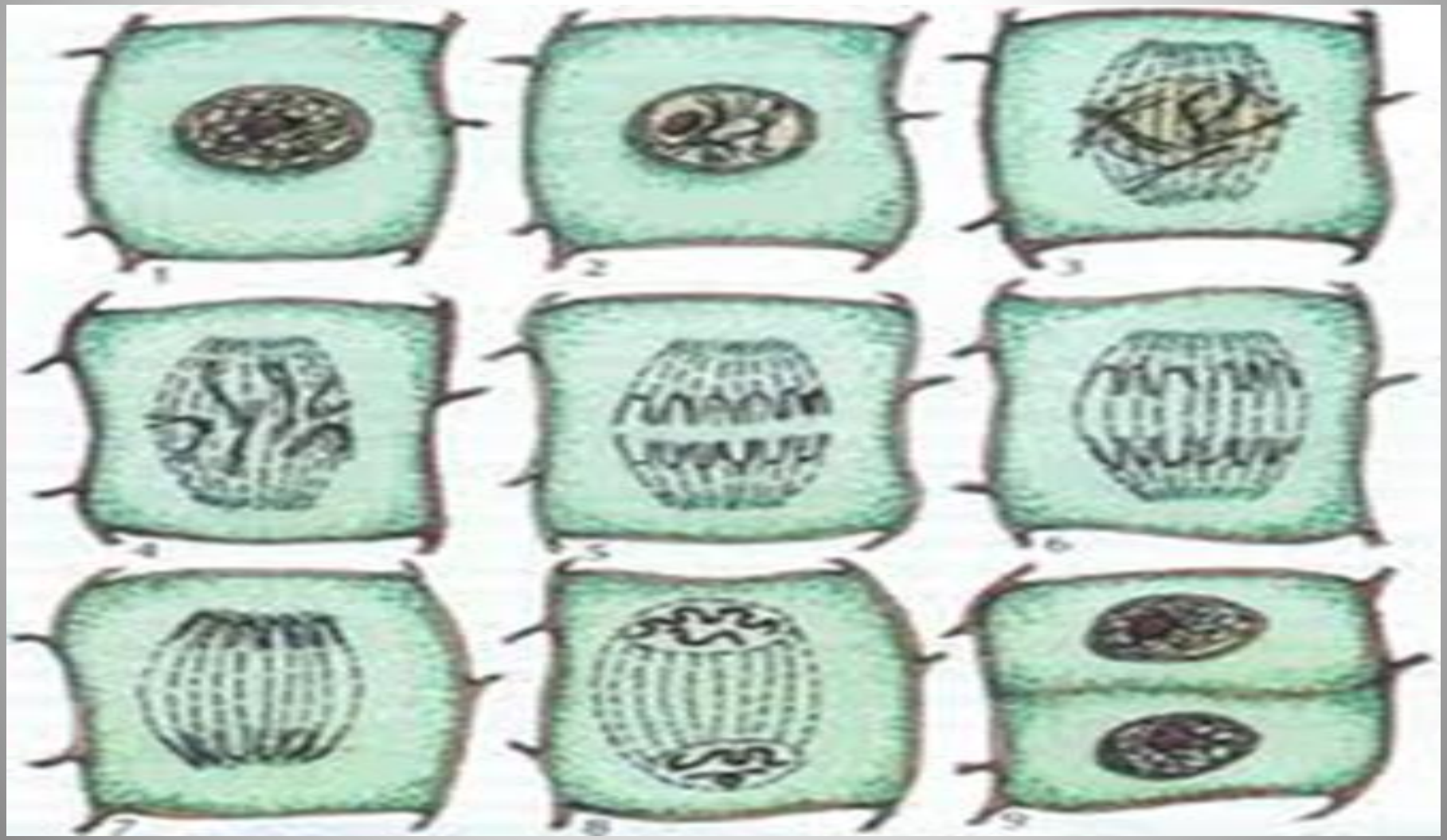
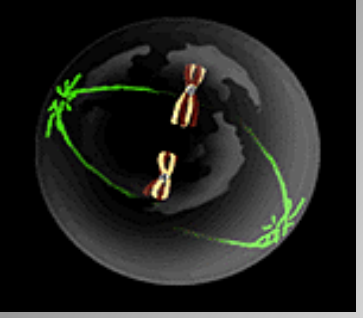


Lecture 4

Life cycle of cells.
Mitotic cycle.
Regulation of the mitotic cycle.
The concept of apoptosis

Mitosis





Plan of the lecture

1.The concept of life cycle of the mitotic cells
2.Characteristic periods of the mitotic cycle
3.Mitosis and its biological significance
4.Types of cell complexes according to mitotic activity. The concept of the mitotic index.
5.Regulation of the mitotic cycle

- **Life cycle of cells -**this is a set of all processes occurring from cell formation till its demise.
- **The mitotic cycle -** a set of processes occurring in the cell during its preparation for division and during division.

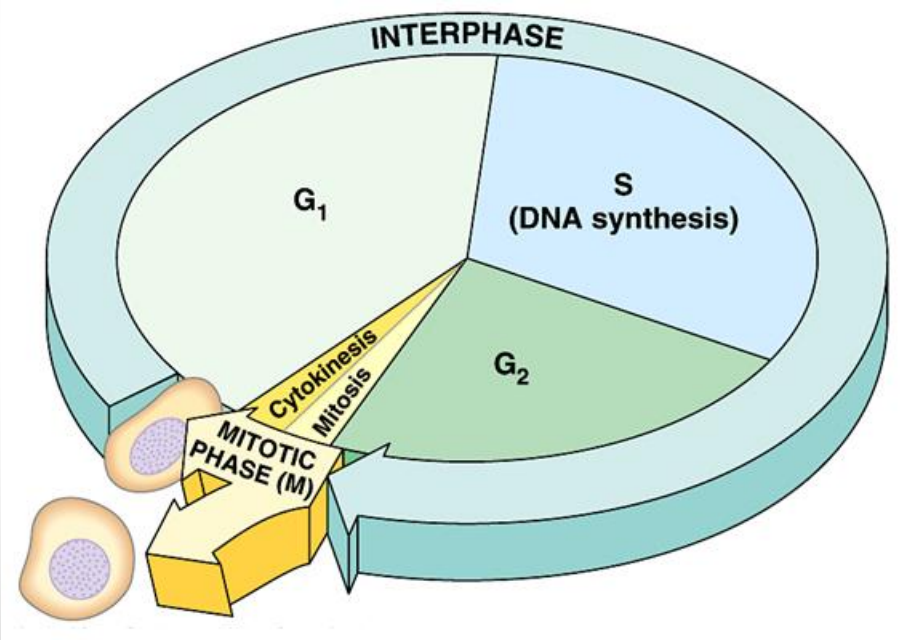
Mitotic cycle

Set of processes occurring in a cell from one division to another division, and division it self are called the mitotic cycle.

Its duration is different for different organisms for bacterial cell cycle may take 20-30 minutes; paramecium caudatum may divide 1-2 times a day.

The cells of multicellular organisms have different ability to divide. If at the early stages of development of the organism, they divide rapidly, the adult organism mostly loses this ability.

Mitotic (cell) cycle The cell cycle is divided into 2 major phases Interphase - between mitotic events. During this stage the cell **grows**, and DNA will ***replicate.*** Mitotic phase - the mother cell divides into two genetically identical daughter cells

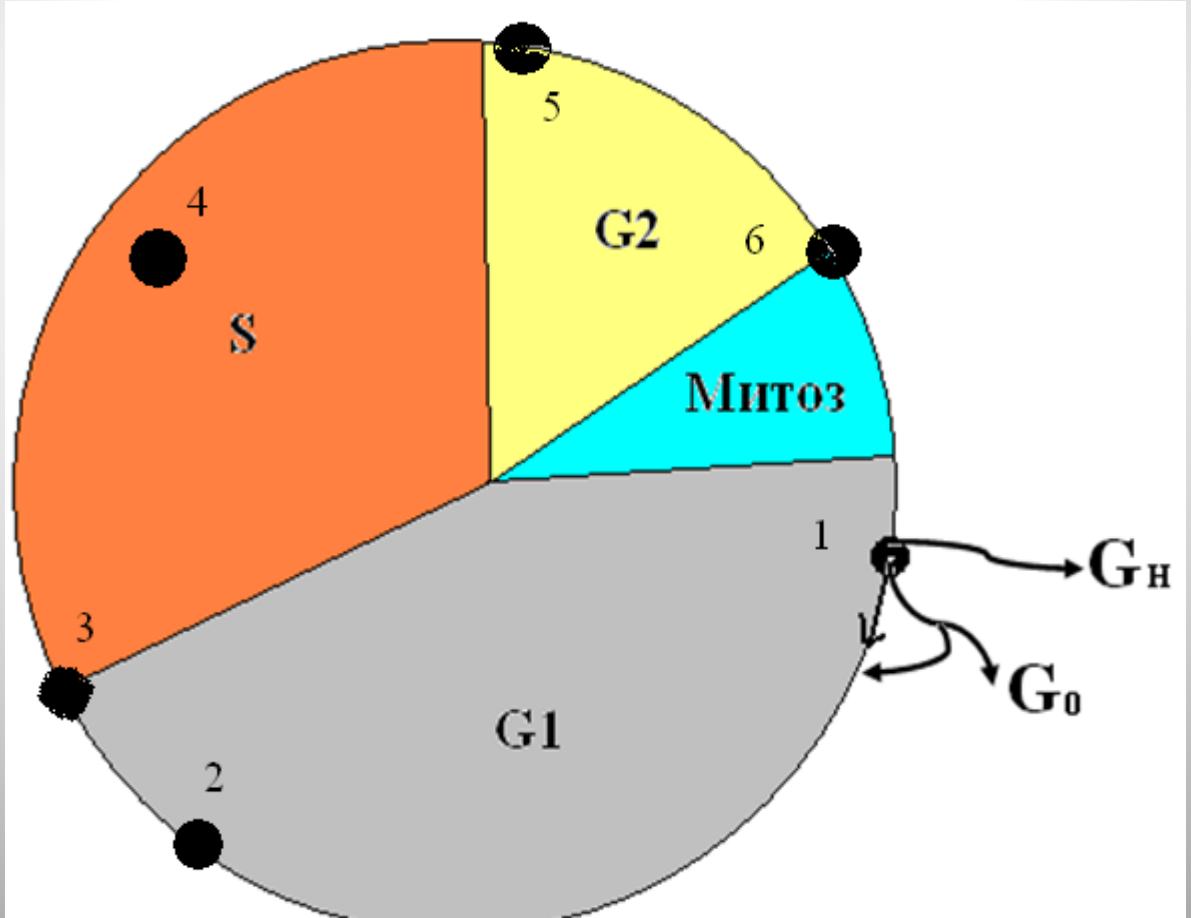


Interphase periods:

* presynthetic or postmitotic - **G1 (2n2c)**

*Synthetic - **S (2n4c)**

* postsynthetic or premitotic - **G2 (2n4c)**



Characteristics of the mitotic cycle periods

- Typical mitotic cycle of eukaryotic cells has interphase and mitosis.
  In interphase there are ***4 periods***:

  1. presynthetic ***(G1),***
  2. during DNA synthesis, or synthetic (***S),***
  3. postsynthetic ***(G2).***
  4. mitosis***(M)***

- ***Characteristics of the mitotic cycle periods***

Presynthetic period G1

- Immediately following the division (after mitosis), and characterized by growth of cells, the active processes of metabolism, accumulation of RNA synthesis of proteins required for the formation of cell structures.

  Cell in this period contains a diploid set of one - chomatid chromosomes.

  This is the longest period, it can take from 10 hours to several days.

S-period

- In the ***S-period***, which usually lasts 6-10 hours, there is the main process - replication (doubling) of the DNA, id est synthesis.
  and continues the synthesis of RNA and proteins, which had begun in the G1-period

In the S- period continues synthesis of RNA and proteins , which had begun in the G1- period :

1.intensive synthesis of histone proteins within the cytoplasm and move to the nucleus where they bind to the newly synthesized **DNA**;

2. by the end of the period, each formed chromosom consists of two sister chromatids joined at the centromere region and close to each other;

3. **rRNA** synthesis , which is usel in **G2-** next period;

4. centrioles double ;it is important, the doubling of the mitochondrial and plastids **DNA** may not coincide in time with **S- period** . It is independent of nuclear **DNA** synthesis ;

G2-period (post-synthetic)

*After a full doubling of chromosomes occurs postsynthetic (sometimes called ***premitotic G2-period.***

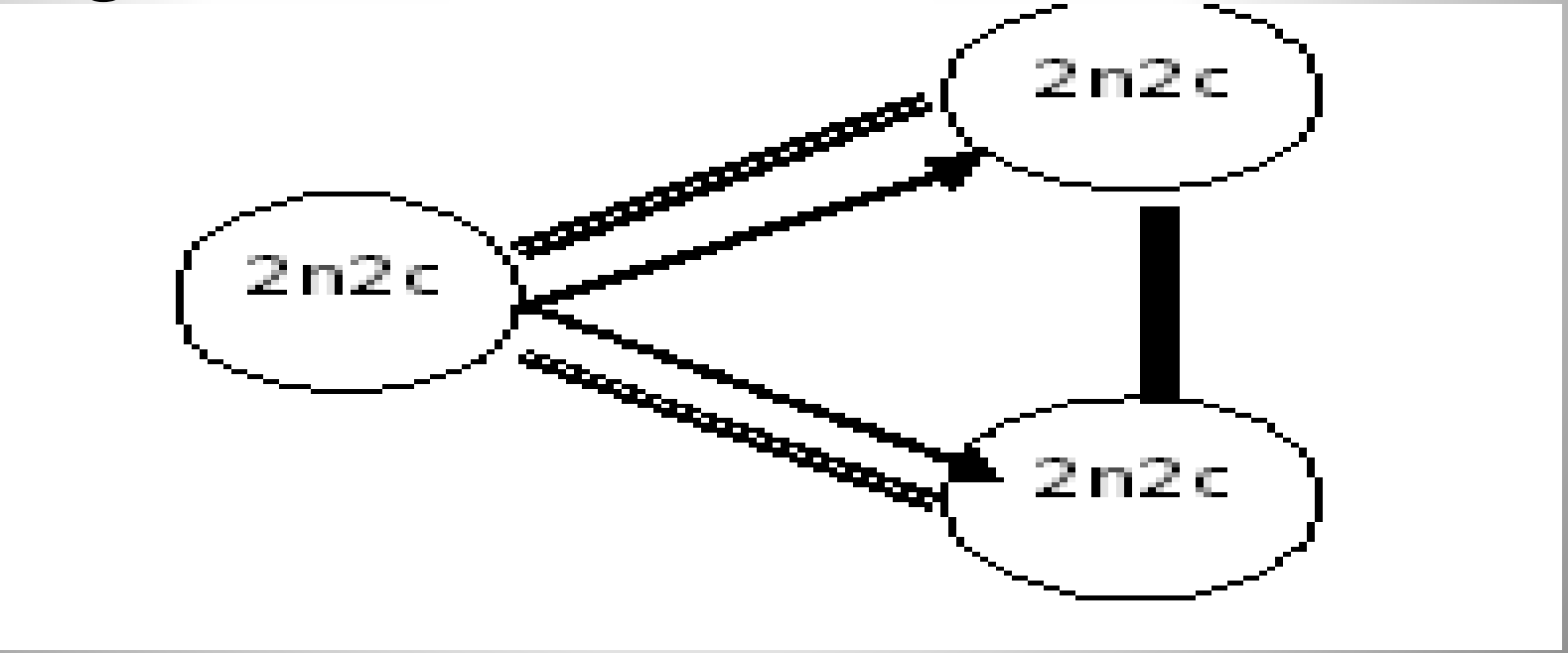
*It continues synthesis of ***RNA*** and proteins (now synthesized proteins of spindle division).

*fibrillar halo around centrioles (animal cells) begins to form .

* Energy is stored.

*The cell in this period contains the diploid set of(***2P***) two-chromatide chromosomes that is the number ***of DNA - 4C*** This period usually takes 3-6 hours, then the cell goes to mitosis.

- **Mitosis is -** indirect division in a euikaryotic cell, at which the accurate distribution of genetic material between daughter cells, each of which receives a diploid chromosome set identical to the original cell.



- **<u>Mitosis involves two processes</u>:**

  I. mitosis

  II. cytokinesis.

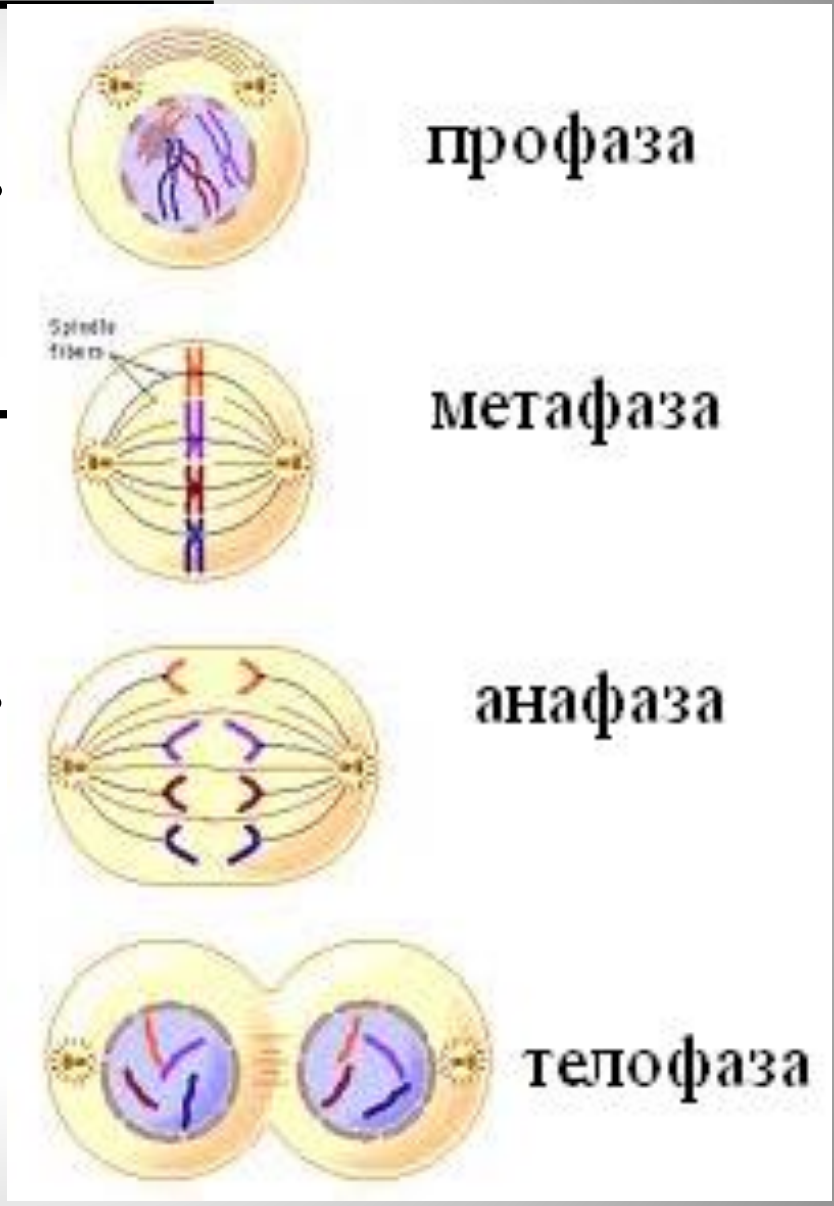
<u>stages of karyokinesis</u>

1.prophase

2.metaphase

3.anaphase

4.telophase



Prophase

During prophase of mitosis the following processes take place:

* **- decay of the nuclear shell into small membrane vesicles (due to the nuclear lamina protein phosphorylation)**
* **- Violation of the stability of the cytoskeleton**
* **- temporal fragmentation of EPS Golgi Apparatus**
* **- nucleoli disappear**

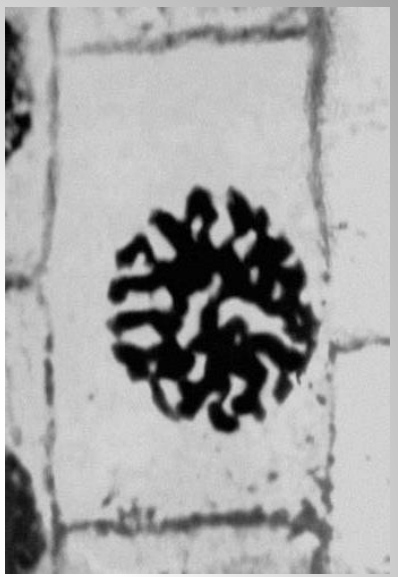
Prophase.

*Chromatin becomes helical/ /begins to be helica/ and /packed/ as a result of that chromosomes are formed. In addition, each of them consists of chromatids, that is diploid chromosome set (2n), and the amount of DNA - 4c)

*By the end of prophase centrioles diverge toward the poles of the cell. Division spindle is formed of two types of microtubules: astral-(away from the centrioles in all directions) and pole-(depart to the equator)

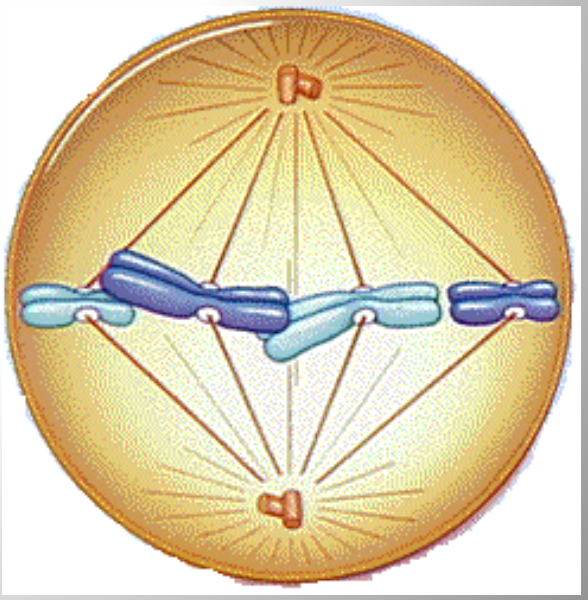
Prophase results:

Helix formation occurs in chromosomes.
Nucleoli disappear,
nuclear shell breaks down.
By the end of prophase centrioles diverge to ward the poles of the cell.
Division spindle formed is formed



Metaphase

- Two-chomatid chromosomes line up along the equator, forming a metaphase plate.
  Spindle threads attach to chromosomes
- Creation of a "parent star" contents of genetic material does not change - 2n4s set of chromosomes.
  forming of chromosomes

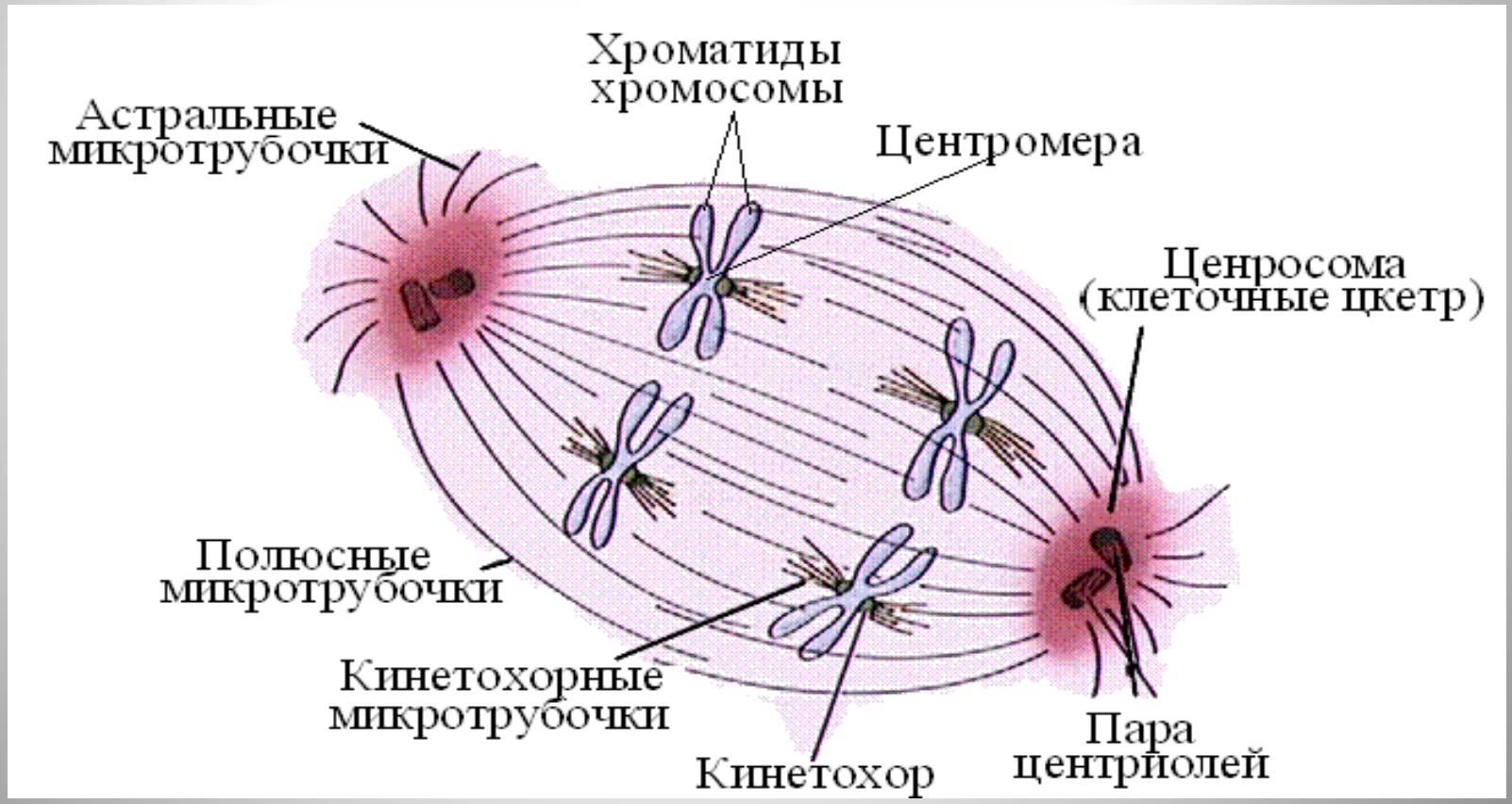


Spindle microtubules

1.kinetochore

2.pole

3.astral



Metaphase

There are early metaphase or prometaphase

* -accession of chromosomes to spindle pole microtubules using protein lamellar structures in the area called the centromere kinetochore, on each chromosome two kinetochore - one for each sister chromatid.
* - chromosomes begin to move toward the equator of the cell;.
* - chromosome become maximum helical
* - Line up on spindle equator, forming a metaphase plate.
* - The content of the genetic material is not changed

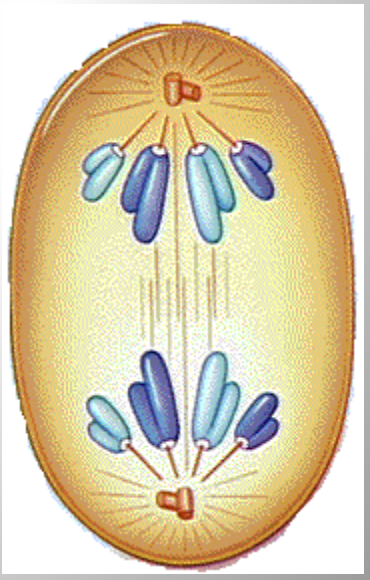
.

Metaphase

- **In animal cells, the chromosomes are positioned so that the centromere regions face the center of the spindle, and shoulders - in the periphery. This position of chromosomes is called the "parent star."**
- **In plant cells, there is no position.**
- **By the end of metaphase the process of separation of sister chromatids completes.**
- **Their shoulders are parallel to each other, visible gap separating them. Contact between them is preserved only in the centromere**

Anaphase

Begins suddenly.
Sister chromatids sinchronieally move away from each other to the ends of the cell.
From this point on, sister chromatids are called daughter chromosomes.
As a result of anaphase cells at different poles are two identical sets of chromosomes: one chromatid diploid set of chromosomes - 2n2s



Anaphase

In anaphase there two phases A and B.

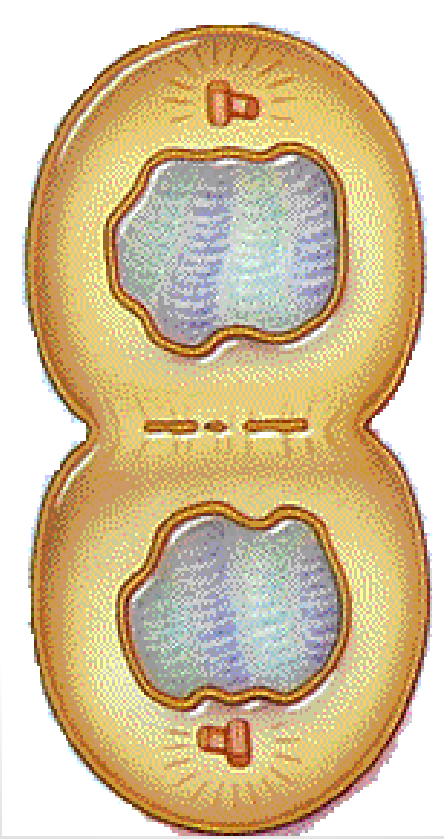
- During ***anaphase A*** chromatid movement is performed by reducing the kinetochore microtubules.

Anaphase

- And in ***anaphase B*** by extension pole and astral microtubules, poles move further cell division that produces more pulling power and promotes divergence chromatids to the poles. From this moment they are called daughter chromosomes.
- ***Anaphase*** - the shortest stage of mitosis (a few percent of the whole time) .
- As a result, at opposite poles of anaphase cells there are two identical diploid sets of one - chromatid chromosomes.

Telophase.

- Telophase processes



Telophase

- It begins with a stop of chromosomes and reconstruction of a new interphase nucleus.
- Chromosomes decondense, increasing in volume. In places of their contact with membrane vesicles of the cytoplasm appears a new nuclear shell. After its closure nucleolus is formed.
- Mitotic spindle (spindle division) collapses.

Cytokinesis

- After telophase cytokinesis usually follows. If it does not, then form multinucleated cells (endosperm plants slime molds plasmodium).
- During cell division in animals constrition is strictly laid in the equatorial plane of spindle. It deepens until two cells are not formed. An important role is played by the cytoskeleton. Cell organelles are distributed rather at random.
- Plant cells are divided by partitions intracellular formation of constriction.

- Thus, as a result of mitotic division occurs exact reproduction of the genetic material and its uniform distribution between the daughter cells, which provides a constant karyotypes of species and genetic succession of numerous generations of cells

Mitosis and its biological role

- Mitosis is the indirect cell division, which is the exact distribution of genetic information between daughter cells. Mitosis consists of two processes - a complex fission **(*kariokinesis*),** and division of the cytoplasm and the actual cells (cytokinesis). Usually this phase takes about 10% of the time the cell cycle.

MEANING OF MITOTIC PROCESS

It has a great positive role for securing useful features and properties in several generations.

- *At the same time mitosis secures negative qualities. Such conservatism prevents evolutionary change.*
- *Mitosis causes the major phenomena of life: growth, development and restoration of tissues and organs, and also underlies the asexual reproduction of organisms*

- Cells of a multicellular organism can be in one of three possible states:
- In the cycle;
- In the resting stage and still able to return to the cycle;
- In the stage of terminal differentiation, in which capacity to divide is completely lost. (Mature neurons, granular blood leukocytes, cardiomyocytes).

Category of cell complexes

There are several different categories of cell complexes, which differ in their mitotic activity:

- Updating cell complexes are presented by not homogeneous sets of cells: **stem, resting, specialized and dead**
- . The body has constantly updated tissues: different types of epithelium, hematopoietic tissues.
- In such tissues, there is a pool of cells that continually divide replacing used or dying cell types (for example cells of intestinal crypts, the basal layer cells of surface epithelium, hematopoietic cells from bone marrow).

- ***Growing cell complexes*** most of the cells are "out of the cycle" in the G0 period - in such complexes there are specialized cells and at the same time there are always cells either in mitosis or ready for it to start

- ***Stable cell complexes*** - neurons and cardiomyocytes - they are characterized by high differentiation and loss of the ability to mitosis; only age changes will take place.

In the body there are also cells which do not proliferate under normal conditions, but they reacquire this property under certain conditions, particularly in need of regeneration of tissues and organs – (***stem cells).***

The mitotic index

To characterize the mitotic activity in the tissues the mitotic index exists - the number of dividing cells per 1000 cells of the tissue.

Mitotic index:

Number of dividing cells /

1000 cells

MI, defines the type of cell complex:

100/1000 - regenerating

10/1000 - growing

1/1000 - stable

Regulation of the mitotic cycle

- The process of cell proliferation is tightly regulated by both the cell (cell cycle regulation, stopping or slowing the synthesis of autocrine growth factors and their receptors) and its microenvironment (no stimulating contacts with neighboring cells and matrix, stop of secretion and (or synthesis of paracrine growth factors).
- Disregulation of cell proliferation leads to unrestricted cell division, which in turn initiates the process of cancer development in an organism.

Regulatory factors that control cell proliferation

- Exogenous factors are in the microenvironment of the cells, and interact with the cell surface.
- Factors which are synthesized by the cell it self and operate within it, are endogenous factors

Factors governing the mitotic activity of the cells

Exogenous factors

Rhythmic factor depends on external factors (rhythm activity, light temperature) and internal (neurohumoral regulation)

Food factor - nutrition stimulates mitotic activity

Stress factor - reduces cell reproduction

Endodenous factors

- Endocrine - samatotropin and thyroid hormones
- Products of tissue breakdown stimulate mitosis and contribute to the regeneration
- Mitogens - accelerators
- Cytostatics - suppressor
- genetic factor

- In the adult human body cells of various tissues and organs are unequal in their ability to divide . Besides during aging rate of cell proliferation is reduced (that is , increasing the interval between mitosis) .
- Back in the 60s of the last century , Leonard Hayflick found that in cell cultures of human somatic cells can divide a limited number of times . While limiting the number of divisions (called the Hayflick limit) strongly depends on the age of the individual , which these cells belong to

- so cells of newborns were divided 80-90 times ;
- 70 -year-olds 20-30 times .
- Reaching the limit cells, go into senescent - senility , which was characterized by an abrupt change in metabolism and is primarily a violation of DNA replication , and then came the death of cells .
- All this made me think about the molecular genetic mechanisms of this phenomenon , that is what induces cell division , specialize and die at the molecular level .

- In 1989 he was awarded the Nobel Prize for the discovery of two types of genes that control cell reproduction:
- Proto-oncogenes accelerators - stimulate mitosis;
- Proto-oncogenes suppressor – suppress mitosis

Proto-oncogenes accelerators

- Encode a family of proteins called cyclin-dependent kinases ***(CDK 1,2,3,4,5,6 -*** Digital index corresponds to the order of their discovery) and ***cyclins A, B, C, D, E.***

Proto-oncogenes suppressor

- Encode another group of enzymes suppressing processes of cell division: ***P13, P15, P16, P53*** and ubiquitin

Restriction points *(checkpoint)*

- In a clear chain of successive phases there are several so-called "points of restriction" after which subsequent events become irreversible. At this restriction point coincide with the advent of cell specific regulatory proteins - cyclins and cyclin-dependent kinases ***(CDK).***
- At different stages of interphase and mitotic cyclins operate various complexes and ***CDK,*** which causes a clear change of mitotic events.

- ***To enter MC*** , cell must obtain a mitogenic signal on the membrane to reach the nucleus.
  Transferring mitotic signal from the cell periphery to its genetic apparatus begins with the activation of growth factors .
  Growth factors (regulators of proliferation) secreted by some cells, and influience the other , which shoud enter into mitosis .
  Growth factors are small proteins that bind with cell surface receptors as a result of wave excitation occurs , ***the so-called transmembrane transport signal transportation***.
- It occurs as a phosphorylation reaction using the enzyme of the proteins which are synthesized under the control of one group of protooncogenes .

- Main function associated with the start of proliferation, takes the plasma membrane of cells, which through receptor molecules perceives a variety of extracellular mitogenic signals and provides transport to the cell substances involvd in division activation

  Cell mitogenic received signal starts the process of dividing.

- To entered cell MC , it must obtain a membrane mitogenic signal to reach the nucleus.
  Transferring mitotic signal from the cell periphery to its genetic apparatus begins with the activation of growth factors .
  Growth factors (regulators of proliferation) secreted by some cells, and act on the other , which should enter into mitosis .
  Growth factors are small proteins that bind to cell surface receptors as a result of wave excitation occurs , the so-called transmembrane transport signal.
  It occurs as a phosphorylation reaction using the enzyme of the proteins which are synthesized under the control of one group of protooncogenes .

Meaning of regulatory mechanisms

- Assignment of cell cycle regulatory mechanisms is not in regulating the cell cycle as such, but to provide, ultimately, the distribution of the hereditary material correctness during cell reproduction.

  The basis of regulation of cell multiplication is changing states of active proliferation and proliferative rest.

Stages of regulation of the mitotic cycle

- ***1. restriction point - R***1 - is the most main moment (period) as "the fate of cells solved" - enter it again in the division cycle or go into a period of rest, and perhaps the stage of terminal differentiation.
  But when the choice is made and the cell enters division cycle, all subsequent steps are performed automatically.

- ***Factor stimulates cells to divide is a protein RUS, which joins cells***

- Mitogenic signal includes first protookogen, which causes the formation of ***complex CDK 2 + CyclinD.*** This complex, in turn, triggers operation of other genes encoding enzymes of DNA replication

  Further, at the end of the presynthetic period (**R2 / S**) work genes encoding ***complexes CDK 4 +CyclinD, CDK5.6 + CyclinD***, necessary for the implementation of pre -replication reparation because body needs cells with normal DNA. ***This corresponds to a second critical point in the cycle - R2.***

S-period (regulatory mechanisms)

- **Starting S-period is** marked by the emergence of another complex **CDK 2 + *Cyclin*A**, which starts work and initiates replication enzymes and actually start **DNA duplication.**
- Then works activator **S** combined with **CDK 2 + *Cyclin*A,** which causes elongation of the synthesis of the DNA molecule.
- At the same time, there are still ***CDK + Cyclin*** that prevent re-replication of **DNA** (their absence leads to the formation politent chromosomes), they prevent premature packing of **DNA** in chromosome

G2 phase (regulatory mechanisms)

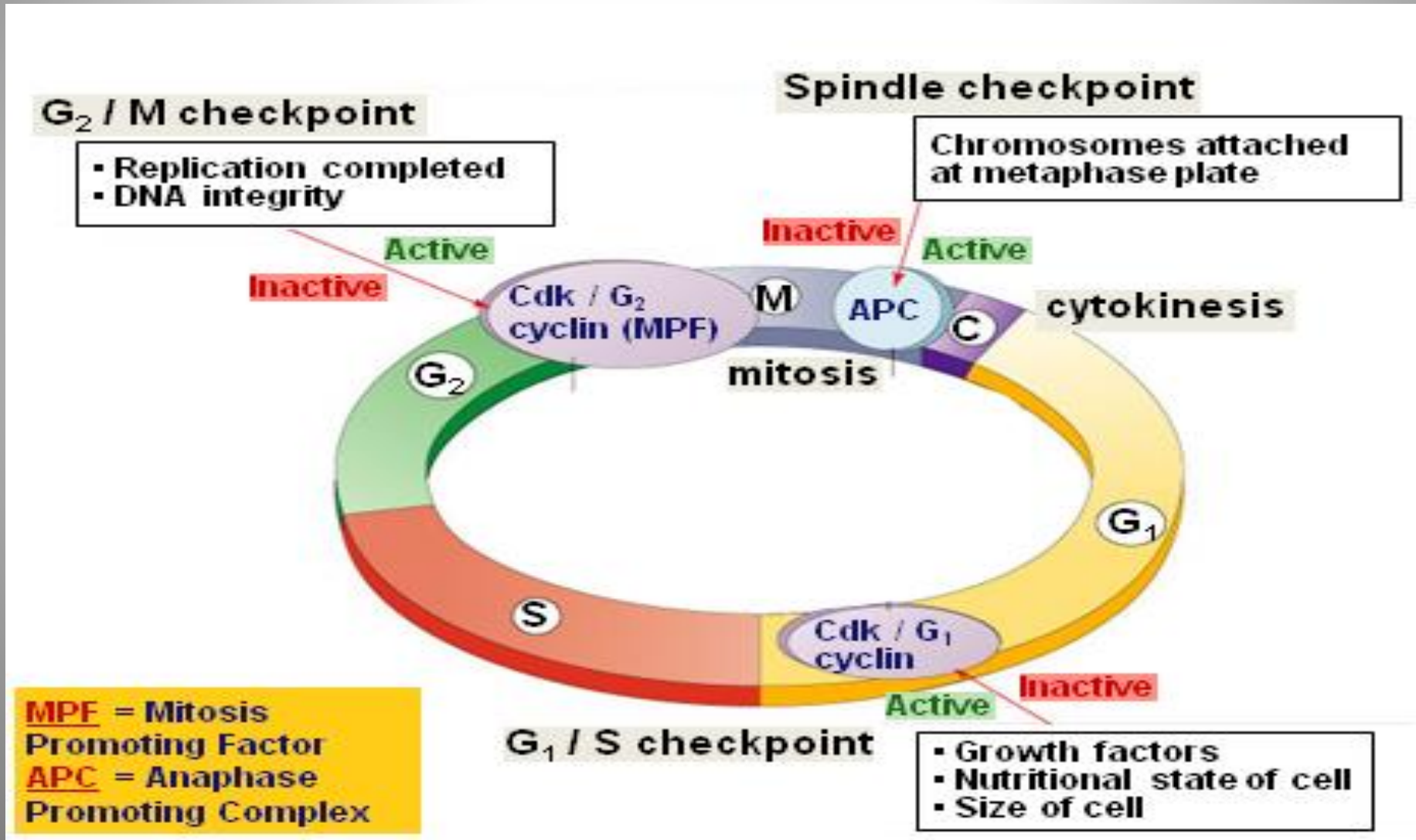
- ***G2 phase*** **-** this phase of the cell cycle, which starts after the completion of DNA synthesis, but prior to its condensation.

  Main regulator passage ***G2 phase is complex Cyclin-CDK2*** **and mitosis stimulating factor (MSF).**

- Regulator of Mitosis is in G2 transition is ***complex Cyclin B-CDK1*****,** its phosphorylation / dephosphorylation regulates entry into M phase**.** ***R3 (G2 / M)***

  DNA damage nonreplicated areals prevent transition to *M phase*

- ***Mitosis*** *is* triggered by the collapse of the complex in mitosis-***CDK1*** and increase by 10 times the concentration of **Ca2 + (Cyclin B** is ***destroyed via the ubiquitin in proteasomes***). Regulation of this phase and the completion of mitosis is carried through the ***IFs*** during its interaction with ubiquitin


G2 / M checkpoint
• Replication completed
• DNA integrity
Active
Inactive
Spindle checkpoint
Chromosomes attached at metaphase plate
Inactive
Active
Cdk / G2 cyclin (MPF)
M
APC
C
cytokinesis
G2
mitosis
G1
S
Cdk / G1 cyclin
Inactive
Active
G1 / S checkpoint
• Growth factors
• Nutritional state of cell
• Size of cell
MPF = Mitosis Promoting Factor
APC = Anaphase Promoting Complex

p53 — master regulator gene