Supplementary materials

Cell cycle regulation

All cells in the adult organism can be divided into three groups according to their ability to divide.

1. Cells that are constantly dividing. We can say that they are constantly in the mitotic cycle. Examples: cells in the basal layer of the multilayer epithelium, intestinal epithelial cells, haematopoietic cells of the initial stages of maturation, spermatogonia.

2. Cells that do not normally divide. As a rule, these are differentiated cells that fulfil certain functions. However, they have retained the ability to divide under the action of certain stimuli. Most often division occurs during the regeneration of an organ or tissue. These cells are in a resting phase (G_0). When certain stimuli are applied, the cells are able to return to the G_1 phase and continue to move through the cell cycle. Examples: liver cells, renal tubule epithelium, smooth muscle cells.

3. Cells that have finally lost the ability to divide. These cells have undergone differentiation, have left the cell cycle and are in the G_0 resting phase. Regeneration can only occur by the intracellular type. Examples: cells of all epidermal layers except the basal layer, nerve cells, cardiac muscle cells, skeletal muscle fibres.

The progression through the cell cycle is regulated by various intracellular and intercellular signals. We will not consider intercellular signals in this material.

Intracellular mechanisms: 1) The cyclin/cyclin-dependent protein kinase (CDK) system 2) CDK activators and inhibitors 3) Proteins encoded by proto-oncogenes and tumour suppressors (anti-oncogenes).

The correct passage of the cell cycle is checked at several control points (checkpoints).

In 2001, Leland Hartwell, Tim Hunt, and Paul Nurse received the Nobel Prize for discovering of the genetic and molecular mechanisms that regulate the cell cycle .

Cyclins are proteins produced at different rates in the cel. The destruction of cyclins also occurs at different rates. Therefore, their concentration in the cytoplasm fluctuates significantly during the various phases of the cell cycle (see figure). Cyclins do not have enzymatic activity. They have a homologous region of 100 amino acids (the so-called cyclin box), which ensures binding to CDK. Therefore, cyclins can form a complex with CDK. The formation of the complex is necessary for CDK activation.

Cyclin D is different from other cyclins. Its concentration increases and decreases gradually and smoothly throughout the cell cycle. The presence of cyclin D coordinates cell growth with the biochemical

processes that occur at the start of a new cell cycle. The concentration of cyclin E increases at the end of the G_1 phase, reaches its maximum at the start of the S phase, and then decreases sharply. Cyclin A levels increase during the S phase, peak during the G_2 phase and then decrease at the beginning of mitosis. Cyclin B content increases during the G_2 phase, reaching its maximum during mitosis, and then decreases sharply.

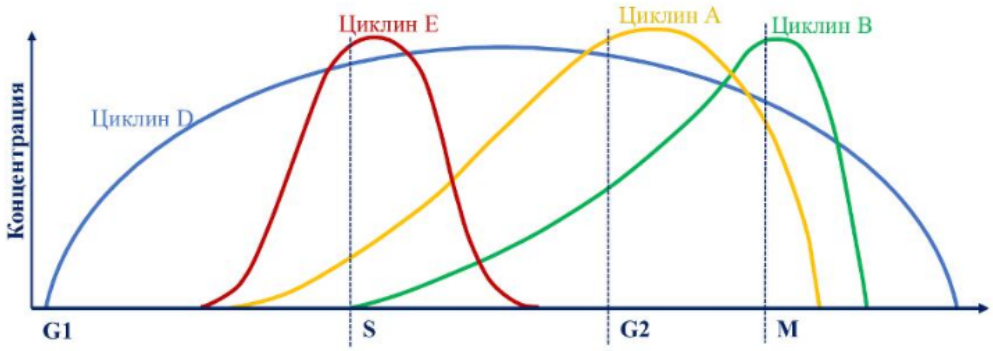
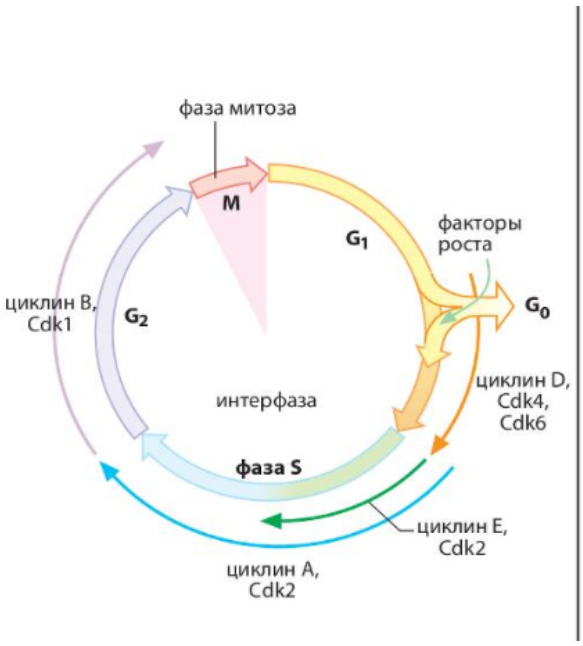


Рис. 11. Флуктуации относительной концентрации циклинов в течение клеточного цикла

CDKs were discovered during yeast research. CDKs are small proteins that are inactive in their native state. Their concentration remains constant throughout the various phases of the cell cycle. However, since the concentration of cyclins varies, it is the level of cyclin that regulates CDKs activity. When the cyclin/CDK complex is formed, the conformation of the enzyme changes, activating the active centre (catalytic site). Active CDKs (or more precisely, the cyclin/CDK complex) then phosphorylate target proteins by adding a phosphate group to them. As a result, the corresponding protein is activated or inactivated.

Cell cycle phase	Complex
G_1	Cyclin D/CDK 4 (or CDK 6)
Transition from G_1 to S	Cyclin E/CDK 2
S	Cyclin A/CDK 2
G_2	Cyclin B/CDK 1 (this complex is also called MPF)
Prophase and metaphase of mitosis	Cyclin B/CDK 1 (MPF)



Cyclin D/CDK 4 (or CDK 6) complex functions at the initial stage of the post-mitotic (G_1) phase, triggering the corresponding intracellular events (e.g. cell growth and protein synthesis for replication).

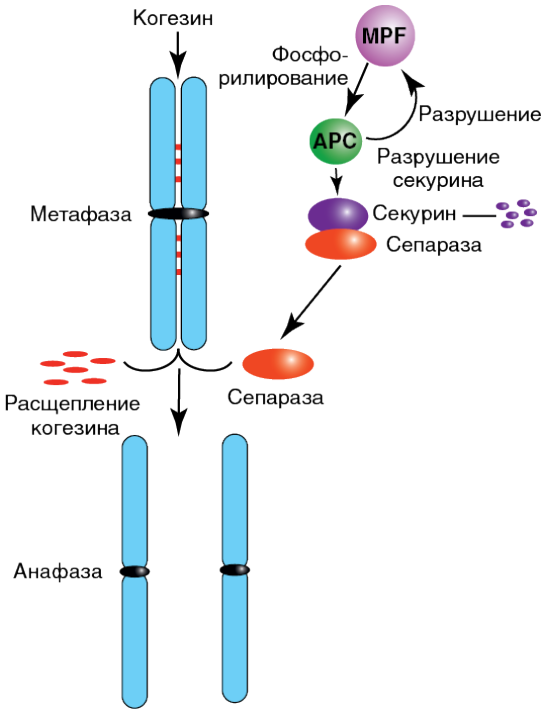
The cyclin E/CDK 2 complex. The cell synthesises a complex of proteins that form the E2F transcription factor. E2F ensures the expression of genes that encode its own proteins, cyclin E, cyclin A, and the synthesis of enzymes necessary for DNA synthesis (e.g. DNA polymerase, ribonucleotide reductase, dihydrofolate reductase). The cell also synthesises the pRb protein. The pRb protein then binds to E2F. When bound, E2F cannot influence gene expression. The cyclin E/CDK 2 complex ensures the phosphorylation of the pRb protein. Phosphorylated pRb changes its conformation and loses its ability to bind to E2F. Free E2F then enters the nucleus and ensures the expression of a number of the above-mentioned genes. As a result, the cell synthesises E2F, cyclin A, cyclin E and enzymes for DNA synthesis, entering the synthetic phase.

The cyclin A/CDK 2 complex acts during the S phase of the cell cycle to ensure that replication (or DNA synthesis) proceeds in such a way that each DNA fragment is replicated once. There are many replication origins on eukaryotic chromosomes, each of which must be bound by the replication enzyme complex only once. The complex (cyclin A/CDK 2) ensures the phosphorylation of replication proteins. This activates the proteins and the replication complex begins to function. In the phosphorylated state, the replication complex cannot rebind to the replication origin.

The cyclin B/CDK 1 complex. A high concentration of this complex is necessary in prophase and metaphase of mitosis, while a low content is crucial in telophase. **The action of the cyclin B/CDK 1 complex**: 1) The integrity of the nuclear envelope is maintained by the nuclear lamina. This is a thin network-like structure formed from filaments that acts as a support

structure. The complex phosphorylates filament proteins, which changes their conformation and the lamina is destroyed. As a result, the nuclear envelope breaks down into small bubbles. 2) The complex also phosphorylates histone H1 and other proteins that support the structure of condensed chromosomes. These proteins then become capable of binding to DNA to form metaphase chromosomes. This ensures the compaction of chromosomes (or, one might say, the condensation of chromatin). 3) The cyclin B/CDK 1 complex also phosphorylates the tubulin protein. This changes the conformation of tubulin , promoting its polymerisation to form microtubules.

The cell contains a complex of proteins called the APC. For anaphase to occur, the cyclin B/CDK 1 complex must be destroyed. During metaphase of mitosis, the complex phosphorylates the APC protein. The phosphorylated APC is then activated, ensuring the destruction of cyclin B and the rapid inactivation of the cyclin B/CDK 1 complex. Sister chromatids in a chromosome are connected by a complex of proteins called cohesin. For the chromatids to separate, the cohesin complex must be destroyed. The enzyme separase is necessary for this destruction. Before anaphase, separase is bound to the protein securin and is in an inactive state. Phosphorylated APC causes securin to be cleaved. This activates separase, which then cleaves cohesin. This allows the sister chromatids to separate (see figure).



Checkpoints

During the cell cycle, the cell monitors its own state. To do this, there are several checkpoints in the cycle where the state of the genetic material is first checked. Depending on the results of the ‘check’, one of the

following options is selected for further action: 1) No damage detected - the cell moves on to the next phase of the cycle. 2) If damage is detected, DNA repair mechanisms (or other processes) are activated to correct the detected defects. If the repair is successful, the cell moves on to the next phase of the cycle. 3) If damage is detected, defect elimination mechanisms are activated. If the defects are irreparable, the apoptosis (programmed cell death) mechanism is triggered.

G_1 period checkpoint. The cycle is stopped if DNA breaks or incorrect chromosome segregation are detected.

S-phase checkpoint. The cycle is stopped in the event of a shortage of nucleotides in the cell, the presence of DNA damage, or incomplete replication.

G_2-phase checkpoint. The cycle is stopped in the event of incomplete replication of any chromosome segments or DNA damage.

Mitosis metaphase checkpoint. The cycle is stopped if the spindle assembles incorrectly or if there are disturbances in the attachment of chromosomes to the spindle fibres.

In most cases, the p53 protein plays a central role in stopping the cycle (see below for more information tumour suppressors).

Proto-oncogenes are normal genes that encode proteins which stimulate cells to progress through the cell cycle. If these genes mutate and their functions are altered, they are called oncogenes. Let's look at a few examples.

Ras is a superfamily of genes? that encode Ras G proteins (these are small GTPases or guanosine triphosphatases). Ras G protein is located on the inner side of the cell membrane and participates in the transmitting an external signal (which stimulates cell proliferation) from the receptor to the nucleus. As a result of mutation, the Ras G protein remains activated, leading to uncontrolled cell proliferation. The mutated ras (oncogene) is found in 15% of all human neoplasms, including 25% of lung cancers, 50% of colon cancers, and 90% of pancreatic cancers.

Erbb2 — (erythroblastic leukemia viral oncogene homolog 2; location - 17q21.1) encodes a protein belonging to the epidermal growth factor receptor family. This protein interacts closely with other epidermal growth factor receptors, stabilising their binding to ligands. As a result, the cell cycle is maintained. Increased expression of the Erbb2 gene occurs in various oncological diseases, such as breast cancer and ovarian cancers.

Anti-oncogenes (tumour suppressors) encode proteins that block the cell cycle. Mutations in oncogene genes invariably result in the formation of a clone of cells that proliferate uncontrollably.

The p53 protein is one of the most important regulators of the cell cycle and is often referred to as the ‘guardian of the genome.’ It is constantly synthesised in the cell, but under normal conditions it is rapidly

destroyed. When DNA (or chromosome) damage is present in the cell, the p53 protein becomes phosphorylated. Phosphorylation of p53 makes the protein stable, and p53 accumulates in the cell nucleus. Active (phosphorylated) p53 acts as a transcription factor for the p21 protein gene. This gene is then expressed, and the p21 protein is produced, which suppresses the activity of various cyclin/CDK complexes (it can be said that p21 is a CDK inhibitor). For this reason, the cell cycle stops, regardless of the phase. If the chromosomes are significantly damaged and their repair is delayed, the active p53 protein accumulates, triggering the expression of a group of genes that initiate apoptosis. The defective cell breaks down into fragments and is phagocytosed by neighbouring cells.

However, in the case of a p53 gene mutation, which results in the absence of a restraining factor, cells with a damaged genome continue to multiply actively, leading to tumour growth. When there are p53 gene mutations on at least one chromosome, the risk of cancer in young people reaches 95%.

The p21 protein family includes three proteins: p21, p27 and p57. These proteins bind and inhibit the following complexes: cyclin D/CDK4, cyclin E/CDK 2 and cyclin A/CDK 2.

The p16 protein is a CDK inhibitor which prevents the interaction of CDK 4/6 with cyclin D.

The pRb protein was first discovered in tumour cells (specifically, retinoblastoma cells) and was initially considered a marker for this tumour. Its role in regulating the cell cycle is described above (see the section on cyclin/CDK complex activity). Loss of the ability of the pRb protein to bind to the E2F transcription factor may contribute to tumour development.

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