LESSON PLAN WITH THE USE OF THE GAME DEGRADATOR

- elementary school (12-15 years)

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The DEGRADATOR game is an educational tool to introduce students at various stages of education to the role of proteins in the cell, their fate and how to regulate the amount of a given protein at any given time. The content information contained in the materials below, as well as in the materials available in the game itself, is extensive and intended primarily for the classroom teacher. The role of the educator is to adapt this content to the level of the audience so that it is not overwhelming for them, but at the same time answers the questions posed by them at their current stage of biology learning. The game levels above 3 go beyond the elementary school material in level (at least in Poland). We propose to use them optionally as a specific example of the general mechanisms governing biology and discuss with them various aspects of the equilibrium prevailing in the cell.

The DEGRADATOR game consists of 10 levels, in each of which the participant faces a different task that deepens their understanding of how the ubiquitin-proteasome system works.

Classroom preparation

The classroom should be equipped with a multimedia projector and a computer to display the presentation and game. Students should have at their disposal a computer, laptop, cell phone or other mobile device with access to the Internet. The handout is expected to last 90 minutes of class time, but due to differences in curricula at different educational institutions, it is possible to conduct the lesson in 45 minutes. The lesson is also adapted for students with special needs who cannot participate in the game. Each stage of the game has been described, and materials can be found in downloadable files in the "For educators" section of the game's website (link on p. 2).

Sample lesson scenario

1. Substantive introduction
2. Solution of levels 1 and 2
3. Collaborative quiz solution at level 3
4. Substantive introduction to levels 4 and 5
5. Solving levels 4 and 5
6. Collaborative quiz solution at level 6
7. Substantive introduction to levels 7 and 9
8. Collaborative quiz solution at level 10

Before starting the activity, the teacher should read the educational materials for the DEGRADATOR game available in the "For educators" section at the following link: https://degradator-game.com, in particular, with the “Great Encyclopedia of Protein Degradation”. The materials contained in the “Great Encyclopedia of Protein Degradation” are also recommended for students, but it is not necessary to read them before participating in the class.

❖ **FIRST AND SECOND LEVEL** - getting acquainted with the rules of the game. *Player moves with the cursor/touch (depending on whether playing on a computer or mobile device) the enzyme - E3 ubiquitin ligase. The goal is to form a four-component complex: ubiquitin - E2 enzyme - E3 enzyme - substrate. The four ubiquitin residues attached to a protein molecule are sufficient signal for its degradation by the proteasome. The player during the first level must correctly degrade four proteins in 3 minutes. In the second level, the player again has to*

reassemble the enzyme complex by directing the E3 ligase, leading to the degradation of four proteins in 3 minutes. However, at this stage, they must remember that the ubiquitin on the E2 enzyme is indirectly renewed in the cell as a result of energy derived from ATP hydrolysis. Therefore, the capture of an ATP molecule enables the regeneration of ubiquitin on the nearby E2 enzyme. Another complication at this level is the appearance of an additional substrate that is not recognized by the E3 ligase.

Before starting the task, we suggest addressing the following topics:

The internal environment of the cell and the role of proteins

At the beginning of the lesson, make sure students remember basic information about the structure of the cell and the composition of the cytoplasm. Particularly important information is that the cytosol is a thick, aqueous solution containing many different types of proteins. Although proteins do not differ from each other to the naked eye, they have a diverse structure and perform a myriad of different functions. Depending on its shape, a protein can be a structural support and maintain the shape of the cell in which it is located (e.g. actin), but it can also act as a **catalyst**, i.e. enable the chemical reactions of its substrates to take place. In the cytoplasmic environment, one of the most important mechanisms enabling enzymatic reactions is diffusion, i.e. the free movement of proteins and their substrates. It should be emphasized that the encounter of an enzyme with a substrate is a completely random event - for this very reason, in DEGRADATOR, all components of the cytoplasm except ligase, which is manually controlled by the player, move chaotically in all directions. Increasing the chance of a given enzymatic reaction occurring can only be done by increasing the concentration of the substrate, enzyme, or raising the temperature (i.e., speeding up diffusion).

Next, make sure students understand the concept of **homeostasis**. Homeostasis is "balance of flows," or in simpler language, a state in which as much of a given thing is consumed as is produced. In a state of homeostasis, there is no overproduction and backlog of excess products, nor is there any deficiency. Every organism and every cell that builds it continually strives for homeostasis of energy, heat, water and,

among many others, protein. When a cell becomes deficient in a particular type of protein, for example, hexokinase (necessary for obtaining energy from sugar – glucose), the molecules of this protein are produced in greater quantity. The vital needs of the cell and the organism it builds are constantly changing, so that after some time the proteins previously produced may no longer be needed. This is one of the situations when such proteins can be destined for degradation in proteasomes, or recycling. Unnecessary or damaged protein molecules are cut into the individual amino acids from which they were formed. The amino acids thus recovered will be reused to build a new, different protein molecule that is more needed at the time.

Nature of enzymes and enzyme complexes

Enzymes catalyze chemical reactions due to their characteristic structure. Students should be sensitized here that in the natural world, physical structure is the sole determinant of function - this is true both at the level of genes and proteins, and at the level of whole tissues and organisms. Enzyme proteins adapt to their substrates by means of the shape of their surface, which they fit into the substrates like a glove fitting into your hand. The site where the enzyme binds the substrate(s) is called the "active center" and is usually located in a depression or groove on the enzyme's surface. Once a substrate molecule is bound by an enzyme, enzymes can cut it into smaller fragments by tightening its chemical bonds or facilitate the formation of new chemical bonds by bringing two substrate molecules together with sufficiently high force. Enzymes are crucial to life on Earth because they act **selectively** (or **specifically**) - this means that one enzyme matches only one set of substrates and can only perform one chemical reaction. Thus, with the amount of individual enzymes, the cell is able to regulate the intensity of the occurrence of various chemical reactions. Enzymes, in addition to their substrates, can also combine with other proteins into so-called complexes, or assemblies. The binding of two or more proteins to each other is, in some situations, necessary for the catalysis reaction. There may be a situation in which one protein in the complex is responsible for cutting the substrate in two, while the other protein determines the specificity - i.e. about which substrate will be cut.

Mechanism of protein degradation in the cell

Special attention should be paid to the fact that the processes of protein synthesis and degradation are in constant balance with each other, and their disruption leads to the development of diseases associated with excessive destruction, or accumulation of proteins.

The DEGRADATOR game focuses on destroying proteins through the ubiquitin-proteasome system (UPS), but this is not the only mechanism to remove proteins. Mechanisms such as autophagy and the export (ejection) of proteins outside the cell via extracellular vesicles play an important role. Information regarding these mechanisms is presented in the “Great Encyclopedia of Protein Degradation" and in a handout for high schools.

The UPS system is the axis of the game. It is a highly efficient, selective and rapid protein destruction system. It plays a key function in the regulation of cell division, apoptosis, tumorigenesis and inflammatory reactions, being responsible for the degradation of 80-90% of proteins in the cell. For discovering the mechanisms of protein degradation by the UPS system, Aaron Ciechanover, Avram Hershko and Irwin Rose were awarded the Nobel Prize in 2004 (read more at https://www.nobelprize.org/prizes/chemistry/2004/popular-information/)

Ubiquitination acts as a molecular "kiss of death" marking proteins targeted for degradation. It is important to note that the mere appearance of ubiquitin on a protein does not necessarily immediately direct the protein to degradation. A single ubiquitination rarely leads to such an effect, and the proteasome usually recognizes ubiquitin chains. Ubiquitination may also have other regulatory functions, such as affecting the localization of proteins inside the cell.

ATP - the universal energy carrier in the cell

It should be noted that protein degradation processes in the cell require energy inputs from the breakdown of ATP. ATP is a universal energy carrier in the cell. To better understand this concept, we can compare it to fuel (ATP), which is necessary for the engine (enzyme) that moves the car (chemical reaction). Students should be especially sensitized to this, because the processes of protein destruction during digestion in the digestive tract do not require the supply of energy. Hence, protein degradation inside the cell is of a different nature than just "breaking" proteins into amino acids. The main difference is the selectivity of intracellular degradation and the non-selectivity of digestion.

❖ **THIRD LEVEL** - short summary QUIZ

During the quiz, player consolidates the knowledge gained by performing the tasks from previous levels.

❖ **FOURTH AND FIFTH LEVEL** - presentation of a modern PROTAC-type drug that enables selective degradation

Player must degrade four proteins in 3 minutes, which are generally not recognized by the game's protagonist. However, by grabbing a PROTAC molecule, our E3 ligase will be able to bind new substrates. In level five, player has different PROTAC compounds at their disposal to ubiquitinate different substrates with the same E3 ligase.

PROTAC-type compound. Visible parts binding to the E3 enzyme (left) and the new substrate (right).

Targeted therapies

The current trend in medicine is targeted therapies, aimed at selectively turning off specific molecules in a pathologically altered cell. The main idea behind targeted therapies is to create drugs that specifically hit the diseased cell, sparing healthy tissues as much as possible. Disabling or degrading defective protein molecules

holds the promise of effective and safer therapy. Many types of targeted therapies are now being introduced into recommended medical practice. Others, such as those based on mechanisms of targeted protein degradation, are being studied intensively for future introduction into medicine.

Among such molecules are molecular glues (molecular glues) and PROteolytic TArgeting Chimeras (PROTAC) chimeric molecules. **Molecular glues are activators of the degradation system so that proteins recognized by the E3 enzyme are degraded more efficiently.** PROTAC molecules, on the other hand, act as adapters so that we can plug a device that has a European plug, into an American electrical outlet. Since E3 ligase is substrate specific (it binds to a specific protein destined for degradation) then to force it to ubiquitinate another protein, we need to use an "adapter." **Therefore, PROTAC changes the substrate of the reaction, but does not affect its yield.** Hence, we can use PROTAC molecules when we want to get rid of a particular protein from a cell. A group of diseases being intensively studied for the therapeutic potential of PROTAC is cancer, in which there is excessive production of proteins that promote tumor growth and invasiveness. The use of PROTAC has the potential to destroy these proteins and slow the growth of the tumor, making it more amenable to conventional treatment.

- **SIXTH – NINTH LEVEL** - fundamentals of enzymatics and control of biological pathways.

Again, player must degrade the indicated proteins within a certain time. However, in this level, they will be hindered by deubiquitinating enzymes, which cut off the attached ubiquitin residues, thus reducing the chance of their degradation in the proteasome. In higher levels, player will become familiar with their inhibitors and the auto-ubiquitination of E3 ligase, another checkpoint of the protein degradation pathway.

Basic topics in enzymatics

Compounds that speed up the work of enzymes are called activators. These include the previously known molecular glues. Compounds that slow down enzyme reactions are called inhibitors. Inhibitors are commonly used in medicine as drugs, for example,

the popular anti-inflammatory drug acetylsalicylic acid (aspirin) is a permanent inhibitor of the enzyme (cyclooxygenase) that is responsible for the formation of the inflammatory reaction. In the game, while solving level 8, player encounters deubiquitinating enzyme inhibitors that "freeze" it.

Biological pathways have multiple checkpoints

Since biological pathways are tightly controlled, we can speak of a certain dynamic balance between ubiquitination and deubiquitination. DeUBiquitinating enzymes (DUBs) are among of such checkpoints. They cut the bonds between the ubiquitin and the protein, so that the protein is not degraded. Like many regulatory mechanisms in biology, they work in equilibrium with E3 ligases, which are designed to attach ubiquitin residues to proteins. In DEGRADATOR, DUBs are our adversary that prevents the protein from being targeted for degradation in the proteasome. Excess DUBs activity can cause disease. In some types of cancer, they can inhibit the ubiquitination of signaling molecules that promote the growth of tumor cells, which drives tumor growth. Nevertheless, they have very important functions during cell division, thus playing an indispensable role in tissue regeneration and body growth, for example. We strongly encourage you to familiarize students with a comic about DUBs that is posted on the game's website (link on the p. 2) in the “For educators” section.

Another checkpoint is the "suicide" of the E3 ligase - **auto-ubiquitination**. E3 ligases can be degraded in two ways: through a self-catalyzed process (auto-ubiquitination) or via modification catalyzed by other ligases. In the context of this mechanism, it is also worth noting the non-proteolytic functions of autoubiquitination**.** An example of such a function is the activation of E3 ligases, which highlights the complexity and multifunctionality of the ubiquitination process in the regulation of protein function.

TENTH LEVEL - DEGRADATOR game summary quiz

The quiz summarizes the knowledge gained during the game and contained in the "Great Encyclopedia of Protein Degradation."

Learning Outcomes

Student:

1. defines the function and structure of proteins in the cell;
2. determines how chemical reactions occur in the cytoplasm and understands the role of the composition of the cytoplasm for its course;
3. explains the concepts of metabolic pathway (protein turnover) with examples;
4. compares selective intracellular protein degradation from extracellular digestion;
5. defines the role and essence of protein turnover in the cell;
6. lists the types of mechanisms for removing proteins from the cell;
7. demonstrates the relationship between the structure of ATP and its biological role, and identifies its role for the occurrence of reactions in the cell;
8. presents the characteristic features of the structure of the enzyme;
9. explains the essence of enzyme catalysis;
10. presents the theory of substrate specificity of the enzyme;
11. shows the ways of regulation of enzyme activity (activation, inhibition);
12. explains the mechanism of negative feedback in the regulation of metabolic pathways (auto-ubiquitination);
13. understands the pathomechanisms of diseases associated with inadequate protein turnover in the cell.