Cellular Cleaners

Dr Agnieszka Podlaska explores processes which regulate the function of proteasome in yeast cells

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While the destruction of molecules is commonly considered to be among the most important cellular processes, for a cell's integrity to be preserved, synthesis reactions must be balanced by precisely regulated degradation

Diverse groups of proteins inside the cells of our body – the basic building-blocks of all living organisms – are subject to continuous transformation, constantly being synthesized and broken down. These processes can be likened to a construction site: much as buildings are erected based on blueprints, the proteins needed by a cell at any given point are synthesized based on genetic information, with individual amino acids being linked up into long chains. Any proteins which are no longer needed by a cell, incorrectly constructed, or damaged will undergo "demolition" back into their component amino acids. This process of protein degradation chiefly occurs via what is termed "ubiquitin-dependent proteolysis." On the one hand this process enables things to be kept orderly within a cell, preventing the accumulation of unnecessary or damaged proteins and thereby protecting the cell against death, while on the other hand it offers a way to obtain smaller, biologically active protein fragments and to recover free amino acids to be used in building new structures. The destruction of proteins also plays a key role in regulating a wide range of intercellular processes. For example, the degradation of certain proteins called inhibitors may activate certain

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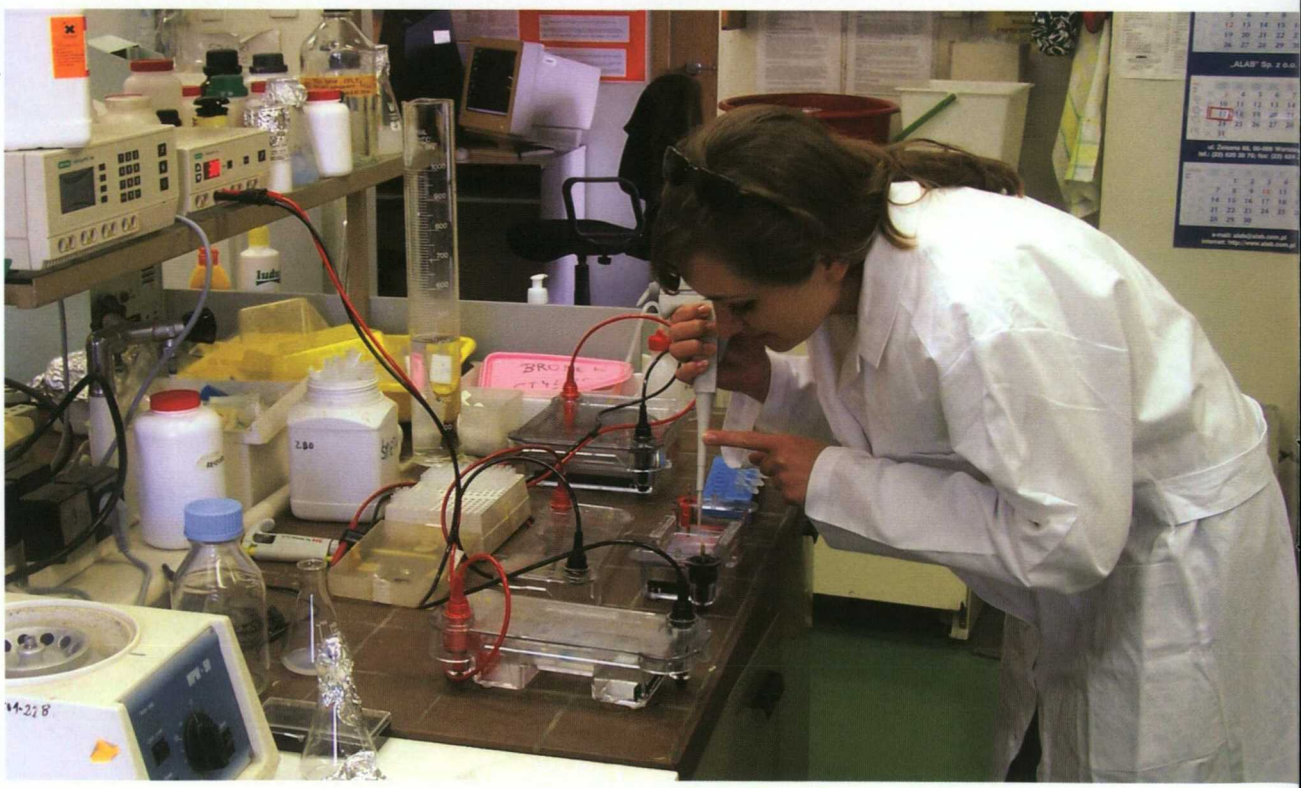

The process of ubiquitin-dependent protein degradation, discovered in the 1980s, is now under study at many laboratories around the world. The 2004 Nobel Prize in chemistry was awarded for work in the field

biochemical pathways in the cell, while proteolysis of an activator protein may act as an "emergency brake," immediately halting a process occurring within the cell.

Miniature spools

How does the ubiquitin-dependent breakdown of proteins work? The process centers around proteasomes, structures reminiscent of miniature spools which act as specialized "protein dismantlers." A human cell contains about 30,000 proteasomes. They are found in both the cytoplasm and the cell nucleus, and their number within the cell changes hinges upon the current need for their destructive action. Very roughly speaking, proteasome is comprised of two multiprotein elements. The first is a core, shaped like a cylinder with a canal leading through it, inside which proteins are broken down. The canal is constructed of four rings, the subunits of which include peptidases - the proteolitic enzymes responsible for protein degradation, or figuratively speaking the "blades" that can slice apart the bonds between the individual amino acids. The other elements of a proteasome are called regulatory caps, situated on one or both sides of the cylinder, whose job it is to recognize proteins meant to be destroyed and to bring them inside the core.

Kiss of death

How does a proteasome recognize the right proteins to destroy? Inside cells there is a certain kind of protein quality control system, whereby specialized enzymes seek out proteins that need to be degraded. Other enzymes mark those proteins, making them easy for proteasomes to recognize, using a special "tag," a chain of tiny proteins called ubiquitin (72 amino acids long) linked together in a specific way. Figuratively speaking, being tagged with ubiquitin is the "kiss of death" for a protein. Once marked in this way, a protein stands no chance of survival. The efficient functioning of both this ubiquitin-based molecular tagging mechanism and the process of protein degradation itself are of crucial imortance for a cell - and not just in terms of eliminating damaged or old proteins. The breakdown of many important proteins, such as p27, p53, and NF-κB, provides a means of regulating such cellular processes as cell division, gene activation, the body's immune response, processes involved in DNA repair, and apoptosis (programmed cell death). Malfunctioning ubiquitin-dependent proteolysis can lead to certain kinds of malignant tumors. Defects in this process are likewise involved in a range of nervous system diseases, such as Alzheimer's and Parkinson's.

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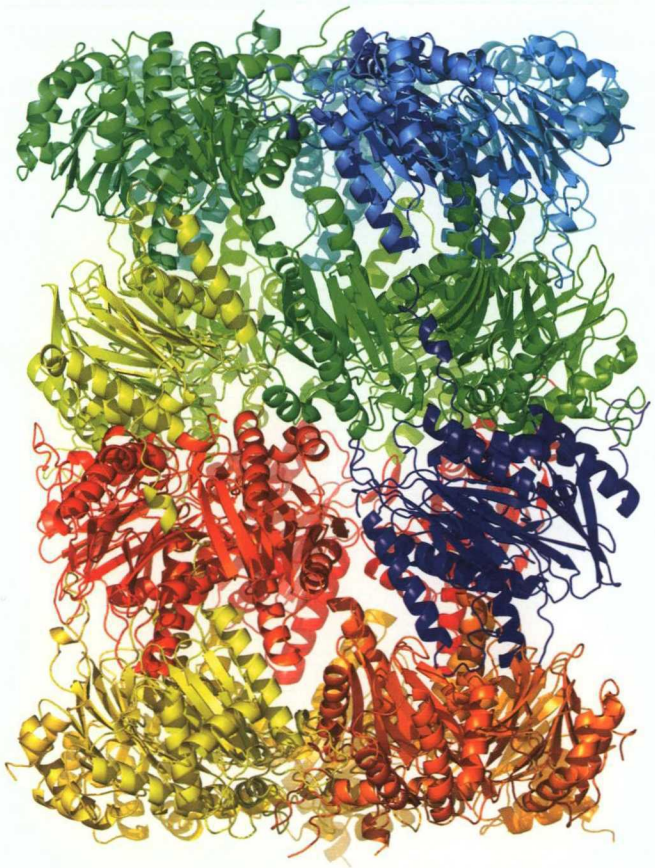
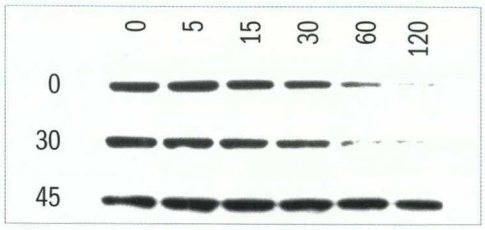


Enzymatic "scissors" function inside the cylindric proteasome core. They break down the chemical bonds of a protein once it is inserted into the channel

The process involved in the ubiquitin-dependent breakdown of proteins was discovered in the 1980s. In view of its complexity and potential impact on a great many cellular mechanisms, proteolysis continues to be the focus of study at many laboratories around the world. Some of this research won two Israelis, Aaron Ciechanover and Avram Hershko, and the American Irvin Rose a Nobel Prize in chemistry in 2004. Since early 2000, at the Laboratory of Mutagenesis and DNA Repair at the Institute of Biochemistry and Biophysics, a team lead by Assoc. Prof. Ewa Śledziewska-Gójska has also been studying proteasome function.

The degradation of proteins



Adrianna Skonieczna

The "special purpose" yeast DNA polymerase, Polη, has a short half-life (as time proceeds from left to right, the black stripe representing Polη fades away). But if damage occurs in a cell (here, after UV radiation), the polymerase's lifespan becomes extended. The figures 0, 30, 45 marking the rows indicate time after UV exposure

Our research has looked into proteasome's involvement in controlling the processes of DNA damage repair, utilizing yeast cells as a model eukaryote. DNA damage occurs as a consequence of numerous external and internal factors. When it does occur, DNA structure abnormalities disrupt the processes of replication, thus entailing the risk of mutations harmful to the cell. That is why a number of mechanisms have evolved to enable such damaged genetic material to be detected and removed.

Tolerating damage

Our team has shown that the proper functioning of proteasome is crucial for the function of one such mechanism, called post-replication repair. This mechanism is special in that it allows the cell to copy genetic material despite the presence of DNA damage. The main replicative polymerase which is generally responsible for copying genetic information is unable to continue working when it comes across damage (an alteration in the DNA structure). This polymerase, together with its accompanying proteins assisting in the replication process, then "fall off" the DNA template being copied, thus halting the synthesis of the new strand. In this case, post-replication repair can allow the cell to survive despite the presence of such damage, enabling replication to be continued and giving the daughter cell a full set of genetic information (albeit containing errors).

One of the main mechanisms of post-replication repair involves what are called alternative polymerases (see also: "Faithful Copyists," *Academia* no. 4/2006). With a somewhat different structure to that of ordinary replicative polymerases, these alternative polymerases are able to tolerate DNA damage, at the cost of being less effective at synthesis - they only piece together short DNA segments. Polymerases ζ (*zeta*, Polζ) and η (*eta*, Polη) are two non-replicative polymerases that function in both human and yeast cells. The basic trait of the former is its ability to continue DNA synthesis even if an error occurs in the newly synthesized strand: the insertion of an incorrect nucleotide. This capability, which regular replicative polymerases in principle do not possess, fixes such an error, leading to the occurrence of a mutation. Yet Polζ can also insert a correct nucleotide when damaged thymine, such as thymidine glycol caused by oxidative stress factors, is found on the strand serving as the template for synthesis. That trait also distinguishes Polζ from replicative polymerases.

Under strict control

Polη, in turn, is able to flawlessly replicate over other DNA damage, such as thymine dimers caused by UV radiation. However, when copying undamaged DNA, it makes mistakes 1000-10,000 times more frequently than replicative polymerases. Both an excess and a shortage of Polη are harmful for a cell. In humans, a mutation in the gene which codes Polη causes a tragic disease known as xeroderma pigmentosum-variant, rendering cells very sensitive to the mutagenic action of UV radiation and drastically increasing patients' chance of skin cancer.

The presence of alternative polymerases in a cell can therefore have a salutary effect in helping to counteract DNA damaging factors. Yet their presence can also be dangerous, since their functioning increases the

Model of a proteasome inhibitor used for treatment of myeloma: light blue spheres - carbon atoms, yellow - hydrogen, green - boron, blue - nitrogen, red - oxygen

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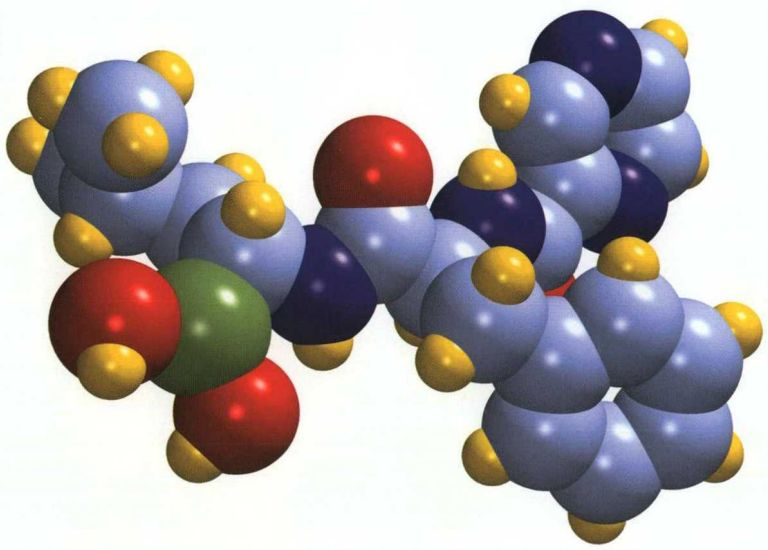
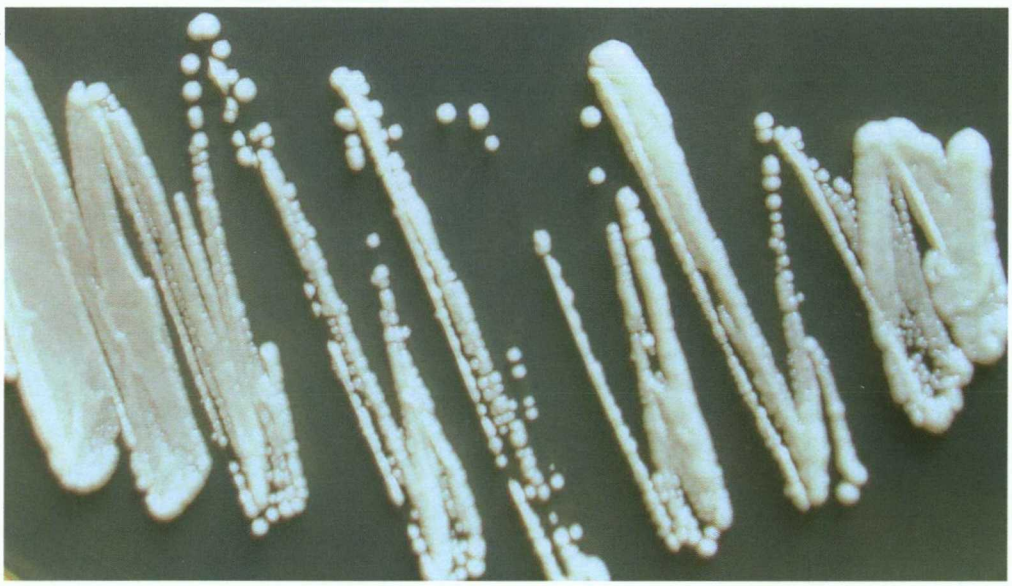


Marta Fikus-Kryńska



Studying simple organisms, such as baker's yeast (*Saccharomyces cerevisiae*), helps us to unravel the complex processes and mechanisms that occur in the cells of higher organisms, including human cells

number of DNA mutations. For this reason, the quantity of such proteins in a cell and their access to the replication forks must be carefully controlled. One method of controlling the quantity of such enzymes, which are "dangerous" albeit crucial in exceptional situations, may involve their purposeful destruction. Indeed, our research results clearly indicate that the concentration of alternative polymerases is controlled in yeast cells by means of ubiquitin-dependent proteolysis.

Perishable molecules

We have demonstrated that Polη, coded in yeast cells by the gene RAD30, becomes "tagged" with the ubiquitin chain and therefore gets broken down by proteasome. We have found the half-life of this protein (the period of time it takes until half of its quantity becomes broken down) to be short, at around 20 minutes. The protein's lifespan turned out to lengthen significantly in response to UV radiation, and so the conclusion is that there exists an active system which enables Polη to avoid degradation at times when a cell is under threat. This is the world's first research result indicating that the proper control of alternative DNA polymerases in eukaryotic cells depends upon correctly functioning ubiquitin-dependent proteolysis.

Our ever-broader knowledge about how ubiquitin-dependent proteolysis regulates various intercellular processes, derived from work pursued at many labs, can be used in the design of new therapeutics. Proteasome inhibitors have been introduced into clinical practice for treating multiple myeloma. Plans call for their application to be expanded to other cancerous diseases, and research is now underway on their use for treating stroke or heart disease. However, since proteasome activity seems to regulate many processes in the cell, inhibiting its activity can cause many side effects. Closer study of how proteolysis functions, therefore, will help us to more fully assess the risk involved in using proteasome inhibitors therapeutically. Such research will also aid in the design of more specific inhibitors that can inhibit precisely targeted cellular processes. ■

Further reading:

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