

The importance of recycling in Alzheimer's disease

By Nico P. Dantuma and Laura Bott

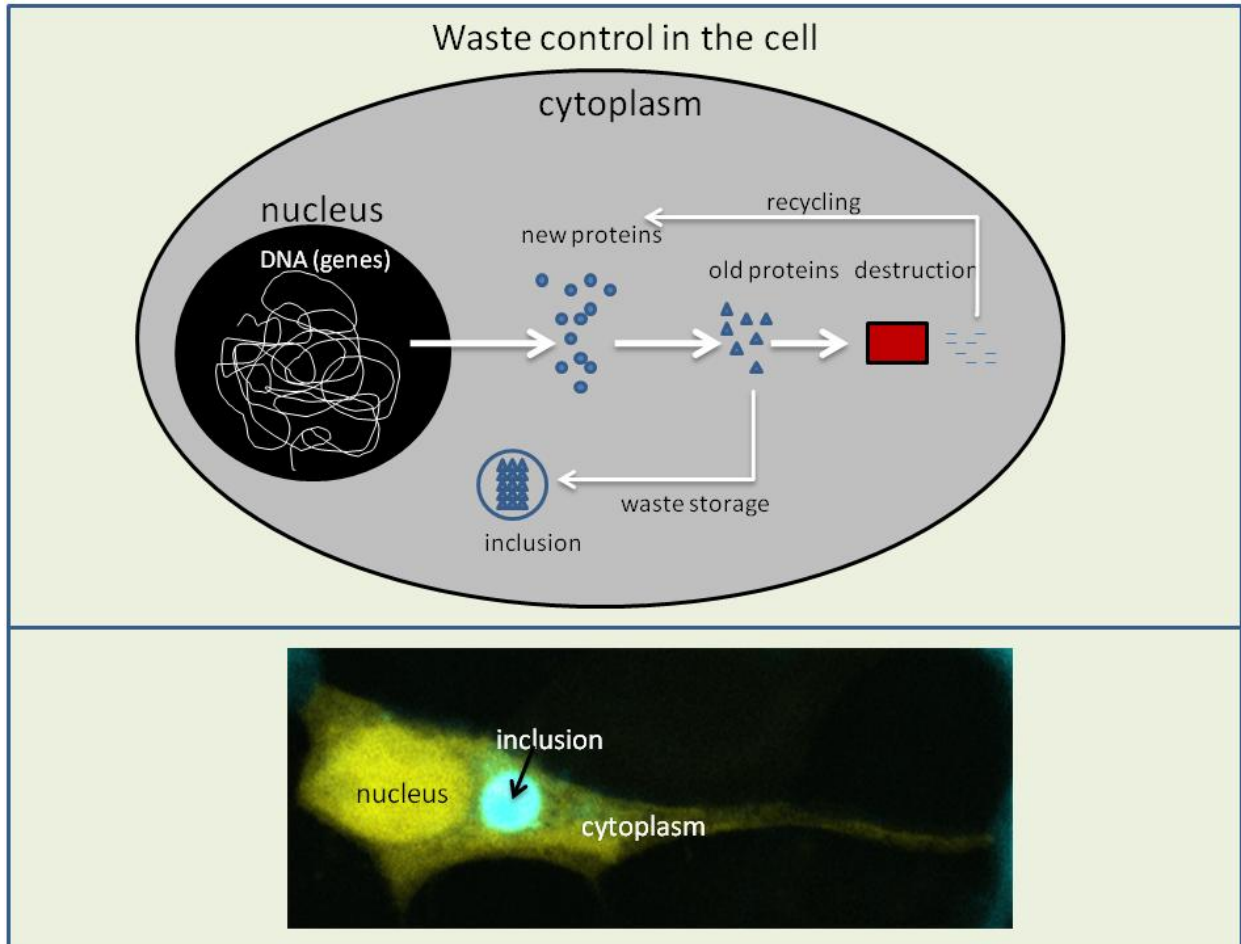
The cells that compose our bodies are absolute masters in recycling. Old, worn-out or damaged proteins are continuously being broken down to their building units which can then be used for the generation of new proteins. Perfect as it may seem, sometimes things can turn ugly. In a number of diseases that primarily concern the nervous system, 'waste' proteins are not being properly handled. Instead of being recycled, faulty proteins accumulate over time and cause serious problems. Notorious examples of so-called neurodegenerative disorders are Alzheimer's disease, Parkinson's disease and Huntington's disease. What happens in these disorders? Why are the proteins not destroyed and how do they intoxicate the cells? These and other important questions relating to protein destruction are a major focus in our research. In order to increase our understanding of the connection between protein recycling and devastating neurological diseases, we have developed tools that allow us to study protein turnover in cells and animals.

Waste disposal in a nutshell

In our everyday lives we may take for granted that our household waste is regularly collected and taken care of by the local authorities, but occasionally we are confronted with situations in which we cannot rely on their services. The pictures of the streets of Pozzuoli filled with garbage during the waste collection crisis in Naples and the reports of a paralyzed city are an uncomfortable illustration of the importance of a fully operative waste disposal system and shows that waste control can be an Achilles' heel in our society. We face similar challenges at a micro-scale in the cells that form our bodies because our cells, too, produce waste. This waste needs to be dealt with such that it does not get beyond control and hinders cells from performing their normal functions.

The genes in our DNA hold the information to build and maintain proteins that fulfill a multi-

tude of actions within the cell. Proteins take care of the energy metabolism, uptake of nutrients, secretion of hormones and many other crucial tasks. Much of the information in our genes is continuously being read by specific complexes and results in the production of new proteins. At the same time, cells have to take care of their old, worn-out or damaged proteins. Since the basic building blocks of proteins can be re-used for the generation of new proteins, "waste" proteins are not targeted for complete destruction. Instead, they are subjected to recycling and therefore the generation and destruction of proteins in cells need to be tightly linked. One may picture that too little protein destruction will lead to pollution of the cellular environment with faulty proteins and this is exactly what we observe in several of the most devastating diseases that affect us human beings.



The upper panel shows the life cycle of proteins in our cells. New proteins are continuously being generated using information contained in our genetic code, while old proteins are broken down and recycled. If protein degradation does not function properly, old or damaged proteins can also be stored in deposits (i.e. inclusions) within the cell. The lower panel shows a magnified cell (yellow) with a single large inclusion (cyan) close to the nucleus

Many of the cells in our body have a limited lifespan after which they are replaced by new cells. A small group of cells, however, cannot be replaced for various reasons. Cells belonging to this latter group are in particular sensitive to waste mismanagement since any imbalance will result in piling up of waste proteins during the many years that these cells exist. One of the cell types that make up our brains and nervous system, so-called neurons, need to last a human lifetime with little or no possibility to substitute corrupted or malfunctioning neurons. It is therefore not surprising that problems in waste

disposal strikes in particular this population of irreplaceable cells since there is no way to compensate for a loss of these cells.

Recycling proteins

But before we turn to diseases, let us first take a closer look at how cells dispose proteins. Central for the destruction of proteins in the cells is the ubiquitin/proteasome system, a complex cascade of events that results in the selective destruction of only those proteins that need to be destroyed. Old and damaged proteins are recognized by a specific class of enzymes called

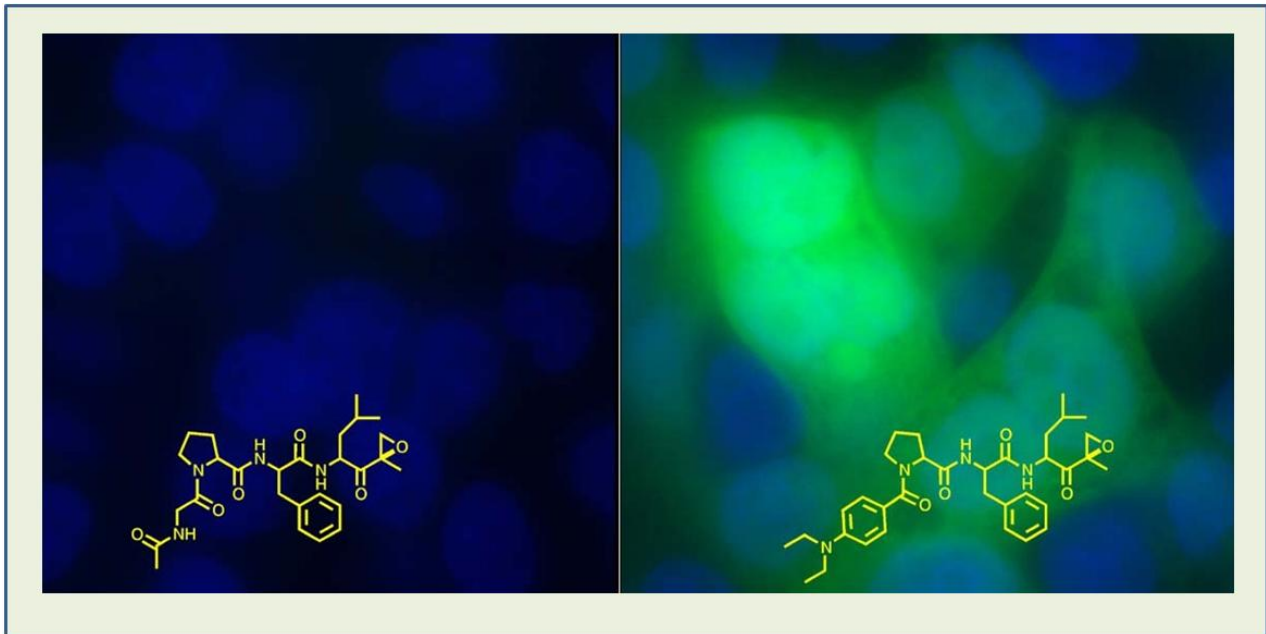
ubiquitin ligases that modify proteins deemed for destruction with ubiquitin, which itself is a small protein. Since ubiquitin targets proteins to destruction and subsequent recycling, it has also been referred to as “the kiss of death”. The final destruction of the tagged protein is executed by large, barrel-shaped complexes termed proteasomes. Proteasomes contain enzymes that act like scissors and chop virtually every protein entering the barrel in small fragments, which, after further processing, can be re-used for the synthesis of new proteins. The process of targeting and degrading cellular proteins by the ubiquitin/proteasome system needs to be tightly controlled and highly specific. In this way we avoid unwanted destruction of our normally functioning protein molecules and ensure that faulty proteins are adequately eliminated.

Alzheimer’s disease and its relatives

A number of diseases that afflict the nervous system have in common that neurons of affected individuals develop deposits of abnormal proteins which have escaped destruction. Collectively known as neurodegenerative disorders, the most notorious examples are Alzheimer’s disease, Parkinson’s disease and Huntington’s disease, but a large number of less known but equally devastating disorders can be added to this list. In each of these disorders, symptoms appear rather abruptly and worsen with age, gradually immobilizing the patient, and eventually lead to death. Even though researchers initially thought that they had found a prime suspect for the diseases in the mysterious protein deposits, it has become clear over the years that deposits are probably the last resort for cells that have failed to keep their environment clean from abnormal proteins. Storing potentially toxic proteins that that may otherwise

pollute the cell in garbage dumps appears to be a good alternative for preserving the integrity of the cell. However, the rescuing effect probably lasts only until amounts of damaged protein exceed the cell’s “caretaker” capacity, and, in many cases, postpones the deadly fate of the cell.

The appearance of protein deposits in a very diverse group of symptomatically different neurological diseases has raised a number of important issues. As mentioned above, it is not surprising that neurons are particularly vulnerable to dysfunction in the protein destruction machinery compared to other cell types. Due to their long lifespan and lack of replacement, neurons, in case of waste mismanagement, will accumulate large amounts of old and damaged proteins and be irreversibly lost in case they do not resolve the problematic situation. However, this leaves the question of what is the initial cause for the failure in protein destruction. The fact that the vast majority of people do not develop neurological diseases shows that our neurons are capable of avoiding failures in protein clearance even at older ages. Is, for some reason, the protein destruction machinery overloaded, or do the proteins that end up in the deposits resist recognition and elimination by the ubiquitin/proteasome system? Likewise, it may be possible that the presence of garbage dumps within cells affect the ability of the neurons to take care of their normal protein recycling, which could explain the exceptional toxicity of faulty proteins. At first sight, these may seem straightforward questions that are easy to address with the present scientific standards but even though two decades have passed since the first description of neuronal protein deposits, many questions remain unresolved. A major problem has been the limited availability of tools to follow protein destruction and to de-



Cells that contain a modified GFP 'waste' protein have been treated with two very similar chemical compounds. The chemical formulation of the compounds is shown in yellow. The chemical compound on the right does not block protein destruction and hence the GFP protein remains undetectable. The related chemical compound on the left blocks protein destruction resulting in cells that fluoresce in green because they accumulate the GFP protein.

termine if the ubiquitin/proteasome system is working properly in cells. The complexity of protein destruction has been a major hurdle that hindered us from studying the functionality of the ubiquitin/proteasome system in these diseases.

Illuminating protein destruction

One main difficulty with following protein turnover is that we cannot see the proteins even when sophisticated microscopes are used. Things would be much easier for us researchers if one could simply watch how living cells destroy proteins in real time but the fact that proteins are very small and more-or-less transparent poses an enormous challenge.

In the early nineties, a fluorescent protein isolated from the jellyfish *Aequorea victoria* entered the scientific arena. It had been known for a long time that this jellyfish fluoresce green light due to the presence of a small protein

which we now know as the Green Fluorescent Protein (GFP). Unlike other proteins, this fluorescent protein can be followed by simply looking with microscopes at the greenish fluorescent light it is producing. A very useful observation was that this protein can produce light by itself and does so even after isolation from jellyfish cells. Thus when this gene was introduced, for example, in mice it resulted in mice that fluoresced green light just like the jellyfish. This opened a lot of opportunities since researcher could use it to visualize any protein that they were studying by simply fusing the jellyfish gene to the gene that encoded their favorite protein. It is fair to say that GFP revolutionized cell biology, which is why its discovery was awarded with last year's Nobel Prize for Chemistry.

In the middle of the GFP revolution, we realized that this little protein could be a great help in our studies on protein destruction. By modifying GFP we were able to turn it into a waste

protein that, shortly after it has been made, is targeted for destruction by the ubiquitin/proteasome system. Cells that encounter problems with handling their waste will start to accumulate the GFP waste protein and it is the GFP that we can directly follow by microscopy as a “reporter” for the fate of faulty proteins. By using the fluorescent signal as readout, we can thus get an idea of how well the cellular waste disposal system is working. Introduction of a gene encoding such a modified GFP in mice enabled us to study the efficacy of protein destruction in different organs like the brain, muscles and liver. The GFP reporter mice simplified studies on protein destruction to a great extent and enabled us for the first time to study protein destruction in the context of human disease.

Lessons from a jellyfish protein

Many laboratories have been using this model to study the intriguing link between neurodegenerative disorders and the protein destruction machinery, and, as often in science, the situation has turned out to be more complicated than we originally anticipated. By breeding mice that have the GFP reporter protein with mice that develop certain neurodegenerative diseases, one can follow the disease course and, at the same time, look at the functionality of protein destruction. In some diseases, such as amyotrophic lateral sclerosis (ALS) and prion diseases (such as Creutzfeld-Jacob disease), it has indeed been found that protein destruction is corrupted but this feature typically appears very late during disease progression when many of the normal processes in the cell also fail to work. Surprisingly, it was found that in several other disorders, including Huntington’s disease, the protein destruction machinery remains operative throughout disease progression. This

observation shows us that the protein destruction machinery is very robust and, at the same time, tells us that there must be other reasons for the cells’ failure to get rid of proteins that end up in the waste deposits. Important in this respect may be the fact that disease-associated proteins tend to stick to each other and often result in tightly packed clumps of protein that may be difficult to handle by the proteasome.

Another twist: protein destruction and cancer

Failure of cells to take care of their protein waste is a serious threat and will lead to improper functioning and eventually death of the affected cell. An interesting and, from a therapeutic perspective, fortunate twist is that cancer cells seem to be even more dependent on protein destruction than healthy cells. This means that chemicals aimed at reducing the activity of the ubiquitin/proteasome system may be potential anti-cancer drugs. In an ideal situation, these drugs should reduce protein destruction to such an extent that they kill the cancer cells but at the same time leave enough activity to keep healthy cells without harm. This delicate balance has indeed been achieved and the first drug based on this principle, known under the brand name Velcade, has recently been introduced into the clinic for the treatment of patients that suffer from multiple myeloma. This type of cancer has been difficult to treat by other means but responds very well to Velcade and a number of clinical trials are in progress to study the effect of Velcade on other types of cancer. Our GFP tools for studying protein destruction are also being used to study the mechanism of this type of anti-cancer drugs and to identify other potential drugs based on the same principle.

The way ahead

The protein destruction machinery in cells is tremendously complex and, even though we have learned a great deal about the ubiquitin/proteasome system during the last three decades, many questions remain. It appears that we are only at the beginning of a long road

with many explorations ahead of us. The link between protein destruction and a broad variety of diseases is not only intriguing but holds also a promise: a promise that more insights will help us with the development of new therapeutics for the treatment of a number of incurable and devastating diseases.

More information

<https://dantuma.cmb.ki.se/dantuma/index.html>

http://nobelprize.org/nobel_prizes/chemistry/laureates/2004/public.html

http://nobelprize.org/nobel_prizes/chemistry/laureates/2008/info.pdf

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