PROF. AGNIESZKA SIRKO

# Preparing for Death

We talk to **Prof. Agnieszka Sirko** from the PAS Institute of Biochemistry and Biophysics about our improving understanding of cellular death.

**Prof. Agnieszka Sirko, PhD, DSc** works at the Department of Plant Biochemistry at the PAS Institute of Biochemistry and Biophysics. Her team studies the role of selective protein degradation in response to abiotic stress in plants, in particular linked to sulfur deficiency. One of the team's achievements is showing that the Joka2/NBR1 protein is a selective autophagy receptor in tobacco.

ACADEMIA: **Do cells die the same way as whole organisms do?**

AGNIESZKA SIRKO: Nothing lasts forever, and this is just as true when it comes to cells – the basic building blocks of all higher organisms. During the lifecycles of multicellular organisms, new cells are formed all the time to replace cells which age and eventually stop performing their designated function due to accumulated damage to their proteins, lipids, or DNA. Cells whose metabolism is not suitably adapted to the organism's current needs are also eliminated. Various environmental toxins, such as metals, can have a direct impact on important cellular elements or have an indirect effect through the induction of reactive forms of oxygen. These forms, known as free radicals, are highly damaging.

Additionally, multicellular organisms must maintain homeostasis – a balance between creating new cells and "killing" off old ones. From this perspective, cell death is a normal physiological phenomenon; for example, during ontogenesis (the period of an individual's origination and development), diametric changes on the metabolic level are frequently needed. It's difficult to achieve such a major "retuning" of metabolism using old, highly specialized cells which are no longer able to divide quickly and which generate many mistakes during cell division. Examples include metamorphosis in insects, the development of mammary glands in mammals, and the formation of xylem – the key transport tissue in plants.

**Tell us more about cell death.**

It may be the result of a number of different processes. One is necrosis, resulting from the degradation of the cell membrane leading to the cell breaking down. It is caused by the destructive action of external factors, and tends to affect many kinds of cells.

A far more complicated process is apoptosis, also known as programmed cell death (PCD). It can be induced by internal or external factors. In a nutshell, the cell shrinks gradually and its cytoskeleton breaks down, while the organelles and cell membrane are preserved. Apoptosis produces cell fragments called apoptotic bodies, and the DNA undergoes a distinctive fragmentation. It should be noted that typical apoptosis is only observed in animals; this is largely due to the specific nature of plant cells, in particular the cell wall which supports cells from the outside. Apoptosis induces cascades of protein proteases known as caspases, whose active center contains cysteine. The enzymes cleave proteins at the site of a specific amino acid: aspartic acid. This leads to the deactivation of proteins key in metabolic process, and eventually to a complete blockade of the cellular metabolism. In contrast to animal cells, plant cells do not contain caspases. Instead they contain caspase-like proteins, such as metacaspases, which perform a similar function by inducing programmed cell death.

However, in the context of cell death we must mention another process: autophagy.

**What's that?**

The term is derived from Greek, and simply means self-devouring. It is a catabolic process, sometimes known as PCD type II, especially in relation to animal cells. I rarely use the latter term myself, since I don't tend to think of autophagy as cell death but rather as maintaining a balance between the processes of synthesis and degradation in cells.

Autophagy is induced by various types of environmental stress, such as abiotic stress (the most notable of which is starvation) and biotic stress (such as viral infection). Just like apoptosis, it is a natural physiological process essential for the correct growth and development of tissues and organs. It reuses building blocks from fragments of cells which are no longer used. Degradation affects different elements of cells, such as protein aggregates, individual proteins, membrane fragments and even whole organelles: peroxisomes, ribosomes, mitochondria or their fragments. Each element is marked and recognized with a high degree of specificity, while the process of autophagy is strictly controlled so that only redundant or damaged elements are degraded, rather than complete cells.

**When we talked about apoptosis, you mentioned a difference between animals and plants. Is there one in the case of autophagy as well?**

Autophagy is a very old, evolutionarily conserved process occurring in the cells of yeasts, animals and plants. To be precise, we talk about three main types of autophagy: microautophagy, chaperone-mediated autophagy and macroautophagy. The latter is the most complex and most heavily studied, and it is what we generally refer to when talking about autophagy. I will continue using the term this way.

> Autophagy – especially in animal and human cells – has become the subject of intensive research in recent years.

Autophagy is a multi-step process. First of all, it must be induced. The best known negative regulator of autophagy is the TOR protein kinase; blocking the enzyme triggers the autophagy process. This property is frequently used in research, where autophagy is induced powerfully by TOR kinase inhibitors. The induction triggers a complicated series of processes which engage various protein complexes at each stage. It should be noted that autophagy involves at least forty proteins, known as ATG proteins. They control each stage of autophagy in turn. The first stage is initiation, involving the formation of a membrane particle known as an autophagophore. In subsequent stages, the double membrane gradually closes around the "cargo" – the cellular fragment to be degraded – which is directed into the vesicle by selective autophagy receptors. The fully closed vesicle is known as an autophagosome. It is then transported using the cellular cytoskeleton to lytic organelles (lysosomes in animal cells or vacuoles in plant or yeast cells). The "cargo" and its receptor are degraded, and the products of the degradation are released into the cytoplasm. Degradation products can be used by the cell for new synthesis processes. Autophagy is a cellular recycling process.

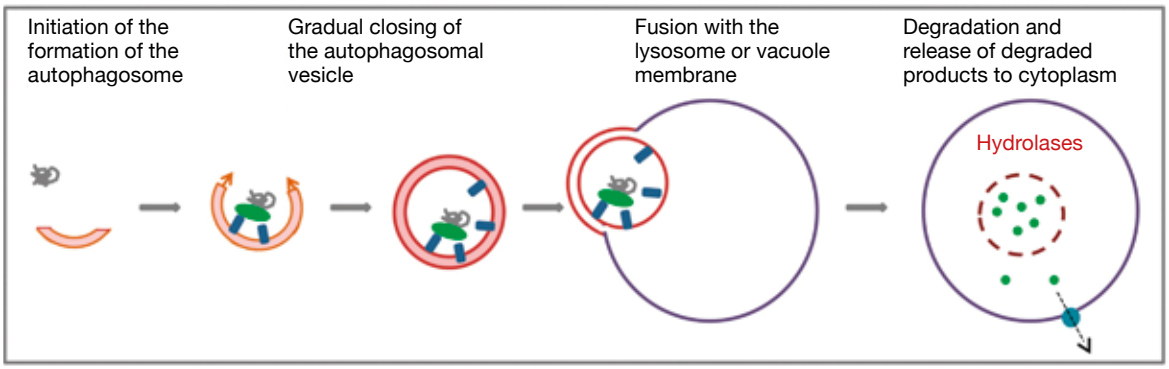
Individual stages of autophagy and the structure of proteins taking part in the process are strongly conserved evolutionarily. This means the process is essential for survival and correct functioning of living organisms, and that it has survived for hundreds of thousands of years in an unchanged form. Naturally there is variation between different kinds of organisms, such as the exact regulatory proteins or different numbers of isoforms of individual proteins. They are more pronounced in plants, since they are immobile and therefore must cope with stress differently to animals. The number of isoforms of different ATG proteins is significantly higher in plants than in animals; yeasts have just one ATG8 protein, humans have six, while the model plant *Arabidopsis thaliana* has nine. The ATG8 protein is an effector of autophagy. It is docked in the autophagosomal membrane where it interacts with autophagy receptors – proteins which bind the "cargo" destined to be degraded.

**What decides whether cells follow the apoptosis or autophagy route?**

Both processes control cellular homeostasis and the turnover of the cell's resources. They are completely independent, although they do affect one another. Generally speaking, autophagy is induced first, which increases the degree of tolerance to stress affecting the cell. Autophagy eliminates harmful or redundant elements, but it can also recycle them for other cellular processes. Autophagy is strictly controlled, which means it usually doesn't lead to cell death. In turn, apoptosis is almost like the next stage, bringing about cell death. I think we need more research to fully understand the mechanisms and relationships between the processes, which will also enable us to develop practical applications.

**What applications are there for autophagy research?**

In recent years, autophagy – especially in animal and human cells – has become the subject of intensive studies. There are many reasons for this. The 2016 Nobel Prize in Physiology or Medicine was awarded to Yoshinori Ohsumi for his contribution to autophagy research. The term was first coined when the process was described in the 1950s; however, it




was only when the genes encoding ATG proteins in yeasts were identified that experimental research of the process became possible. This breakthrough was made by Ohsumi in the early 1990s. This step made it possible to move from passive observations of the process to genetic studies. It has also led to the identification to ATG genes and proteins in other organisms.

Another main reason for our interest in autophagy is the discovery that disorders of the process are implicated in many human diseases, mainly neurodegenerative disorders and certain cancers. Autophagy plays a key role in the immune response, inflammation and during infection. I should add that certain viruses have learned to "manipulate" autophagy processes in their host for their own benefit. The significance of autophagy in healthy and diseased cells is a major driver for research and funding. The number of scientific publications on autophagy is growing rapidly, which translates into preclinical and clinical studies into the process and its role in disease.

**Which aspects of autophagy does your team work on?**

Our understanding of autophagy in animals and humans is developing rapidly, although we still know little about the process in plants. I investigate autophagy in plant cells, or – more precisely – I study the role of proteins which make autophagy a selective process. The proteins are known as selective autophagy receptors. Their distinctive feature is that they bind both the "cargo" destined for degradation in the autophagosome and the ATG8 proteins docked in the autophagosomal membrane which enable the specific delivery of the "cargo."

One such protein in plants is NBR1, related to animal proteins p62 (known as sequestosome 1, SQSTM1) and NBR1. The plant protein is a hybrid containing elements from both the animal proteins. In 2011, we published results of our research in which we successfully identified the Joka2 protein in tobacco in the prestigious journal *Autophagy*. The protein is an autophagy receptor and a homologue of mammalian proteins p62 and NBR1. Until we published our research, it was widely believed that selective autophagy doesn't occur in plants. Research conducted by my team and the identification of proteins acting as selective autophagy receptors in tobacco and in *Arabidopsis*, shown by researchers in Norway, has completely changed our thinking.

We are now mainly working on identifying regulators of the plant NBR1 protein. We are also studying the effect of regulators on the identification of proteins which are due for degradation following binding by the NBR1 receptor. The process is never random, and the role of NBR1 in the precise regulation of cellular homeostasis, including protein homeostasis, is key. In plants it mainly manifests as a response to different kinds of environmental stress, when cellular metabolism must be rapidly reprogrammed. Our focus is currently limited to basic research, but we hope that our work will find practical applications in agriculture and environmental protection.

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