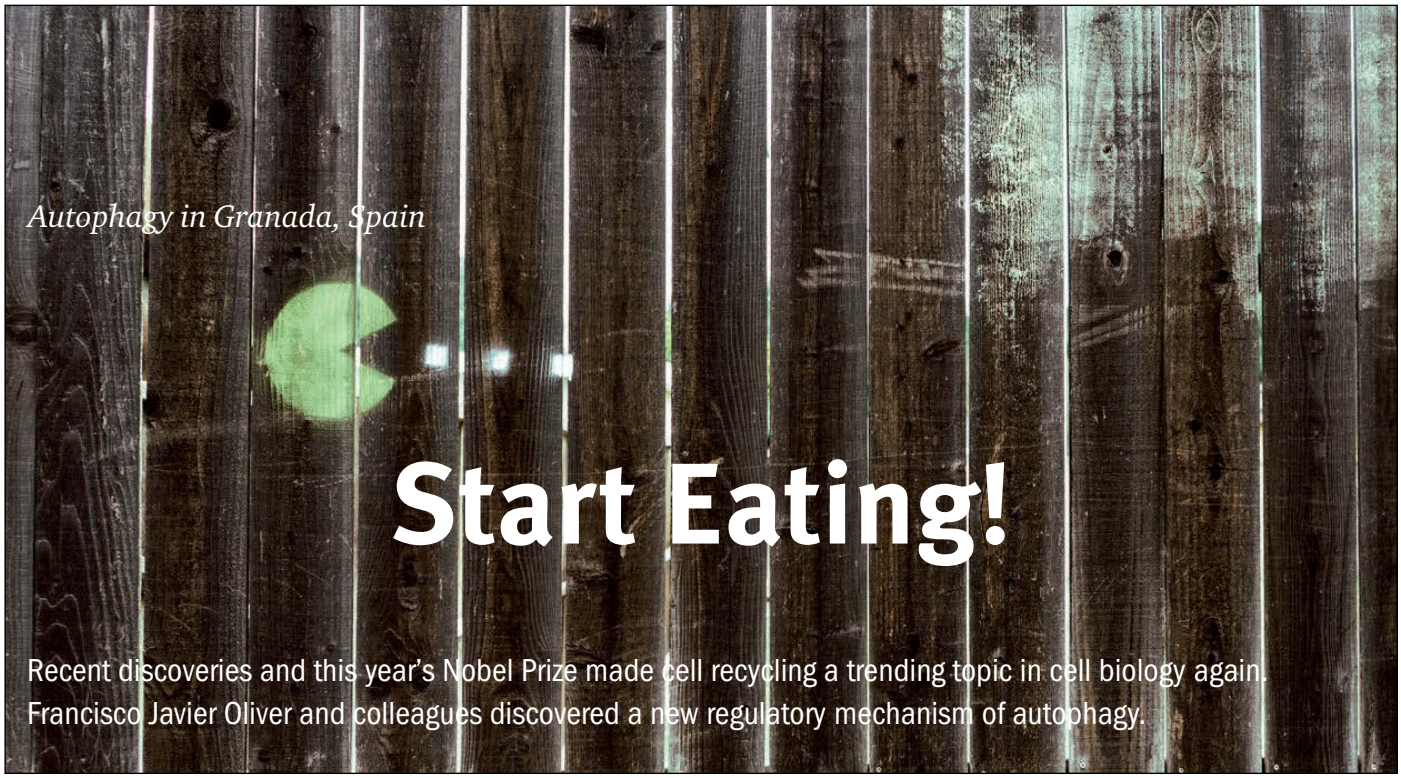


Picture: Jay Mantri

Start Eating!

Recent discoveries and this year's Nobel Prize made cell recycling a trending topic in cell biology again. Francisco Javier Oliver and colleagues discovered a new regulatory mechanism of autophagy.

The word autophagy originates from the Greek words *autós*, meaning "self", and *phageîn*, meaning "to eat, devour". Therefore, autophagy denotes "self-devouring". The term was coined in the 1960s, when researchers recognised that some animal cells could destroy their own content by enclosing it in membranes. These sack-like vesicles were then transported to the lysosome to undergo degradation. And at this point, the knowledge stopped. Scientists knew that the process was especially active when the cell was under stress, due to a shortage in nutrients, for example. But how this process worked and which cells used it, remained a mystery.

And that's where the elaborate work of newly-minted Nobel laureate, Yoshinori Ohsumi comes into play. In the early 1990s, he discovered that baker's yeast also underwent autophagy. As yeast is an excellent tool for genetic analysis, he was able to identify, relatively quickly, many genes essential for autophagy. His subsequent work shed light on the underlying mechanisms of this sophisticated machinery in yeast, and he showed that very similar genes controlled this process in animal and plant cells.

Opening doors

His work opened the door to understanding how the cell recycles its content and the importance of it in many physiological processes. We now know that autophagy plays a crucial role in embryo development, cell differentiation and the immune system. Moreover, a faulty autophagy system has been linked to a wide variety of diseases, including cancer, type 2 diabetes and Parkinson's disease.

All the more important to understand the intracellular mechanisms that spatially and temporally fine-tune the initiation of autophagy. In a recent publication in *Cell Death and Differentiation*, Francisco Javier Oliver from the Spanish National Research Council and colleagues show that decontrol can actually start this controlled cell death (2016 Sep 30. doi: 10.1038/cdd.2016.80).

Three years of work

In a series of experiments that took them three years to complete, they show that for the cell to initiate autophagy, a certain communication pathway between the nucleus and the cytosol has to be established. And the key players in this communication process are AMPK, PARP-1, and the PARP-1/AMPK complex.

Continuing the legacy of Nobel laureate Yoshinori Ohsumi: autophagy researchers Francisco Javier Oliver (right) and his team.

"AMPK (AMP-activated protein kinase) is an enzyme that is essential in maintaining intracellular homoeostasis. It is well known as an energy sensor, linking extracellular milieu fluctuations with the autophagic machinery," says Oliver. PARP-1 (PolyADP-ribose polymerase-1), on the other hand, is an enzyme involved in the regulation of DNA damage- or starvation-induced autophagy. During a process called PARylation, it catalyses the conversion of NAD^+ to polymers of Poly(ADP-ribose) or PAR. And lastly, the PARP-1/AMPK complex is, as its name suggests, a molecular complex formed by PAR-1 and AMPK, residing in the nucleus of non-starved cells. Let's go through them again, in context, one-by-one, starting with PARP.

Studying traffic

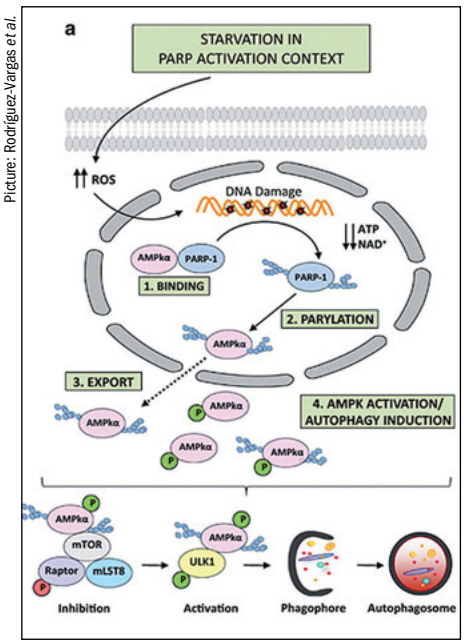
Using a breast cancer cell line, Oliver *et al.* first set out to analyse the importance of PARylation in starvation-induced autophagy. By treating some cells with PARP inhibitors, they could correlate PAR production with starvation. "Cells treated with these PARP inhibitors showed a reduction in autophagy upon starvation. Thus, less PARylation meant less autophagy," Oliver explains. To demonstrate that this is an active process, the scientists studied the cells' membrane traffic. When they treated the cells with autophagy inductors, the rate of autophagy was recovered. Their conclusion: starvation-induced PARylation is directly and actively involved in the initiation of autophagy.

"Next, we investigated how AMPK was involved in this, as AMPK is a well-known positive regulator of autophagy: it is an energetic sensor activated under energy depletion," Oliver says. Following starvation, the scientists saw a time-dependent decrease in ATP levels, correlating with an increase in AMPK activation. Using the same cell lines as before, they found that PARP-1 is also activated during nutrient deprivation. Cell lines treated with PARP inhibitors, however, did not show this starvation-induced ATP

depletion. Moreover, AMPK activation was delayed in cells unable to produce PARP-1. The functional link between PARP-1 and AMPK had been made: PARP-1 is needed for the adequate activation of AMPK and thus starvation-induced autophagy.

There was only one more link to be made: how do these two enzymes interact? The answer proved to be quite straightforward, when using a technique called co-immunoprecipitation to confirm the researchers' suspicion. And indeed, by pulling out the entire protein complex to identify its members, they found that PARP-1 and AMPK form a molecular complex in the

Picture: Rodriguez-Vargas *et al.*



PARylation regulates autophagy through AMPK activation. More details in the article.

nucleus of non-starved cells. "Further immunoprecipitation showed that starvation-induced autophagy causes the PARylation of this complex. When PARP was inhibited, the AMPK could not be PARylated upon starvation, and this coincided with a stabilisation of the PARP-1/AMPK complex," Oliver points out. Or in other words: PARP-1 activation leads to AMPK PARylation and thus dissociation of the PARP-1/AMPK complex in the nucleus.

The last set of experiments focussed on the question how this signal is conveyed to the cytosol, where most of the autophagic pathway takes place. "When measuring the levels of total and activated AMPK, we found that the level of nuclear AMPK declined after starvation: it was transported to the cytosol where more AMPK was subsequently being activated," the scientist observed. The story was complete. Upon starvation, PARP-1 catalyses PARylation, which induces the dissociation of the PARP-1/AMPK complex, leading to the export of free PARylated nuclear AMPK to the cytoplasm, to initiate the cytosolic part of autophagy.

Enzymes in the classroom

Can this complicated process perhaps be summed up in more simplistic terms? Francisco Javier Oliver agrees that a classroom metaphor would do the trick: PARP-1 is the 'teacher' that holds back her 'pupils' (AMPK) from running outside and activate their 'friends' on the playground to deconstruct their playing objects. "But be aware," he says, "the teacher (PARP-1) also requires the strict control of the pupils (AMPK). Otherwise she will produce a metabolic catastrophe in the cell by consuming most of the cell's energy storage."

Oliver's group is keen to start working on the follow-up questions of this paper. These range from finding out how other PARP family members are involved in the PARylation activity, to an even better understanding of the PARP-1/AMPK complex.

In general, he believes that autophagy research in the coming years will focus on the intricate connection between autophagy and metabolic disruptions, such as obesity. Moreover, tumour biologists are currently wondering, whether autophagy can drive tumour malignancy due to its selective cell death. And all this as a legacy of Yoshinori Ohsumi's research in the 1990s that revealed the fundamental importance of autophagy in physiology and medicine.

Nobel Prize = more funding?

"I was really excited about the news of this year's Nobel Prize in Physiology or Medicine. Yoshinori Ohsumi has built up the molecular fundaments of autophagy, from which all cell biologists but, for example, also neurobiologists have benefited to delineate the acting of this mechanism in mammalian cells. Autophagy is essential in the process of aging and a distorted mechanism has severe consequences, as can be seen in the wide variety of diseases, with which it is involved. [...] The fact that the media is now talking about this topic will enhance the possibilities, to attract research funds to study this fundamental process and its implications in a variety of human pathologies," Oliver concludes.

Hedwig Ens

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