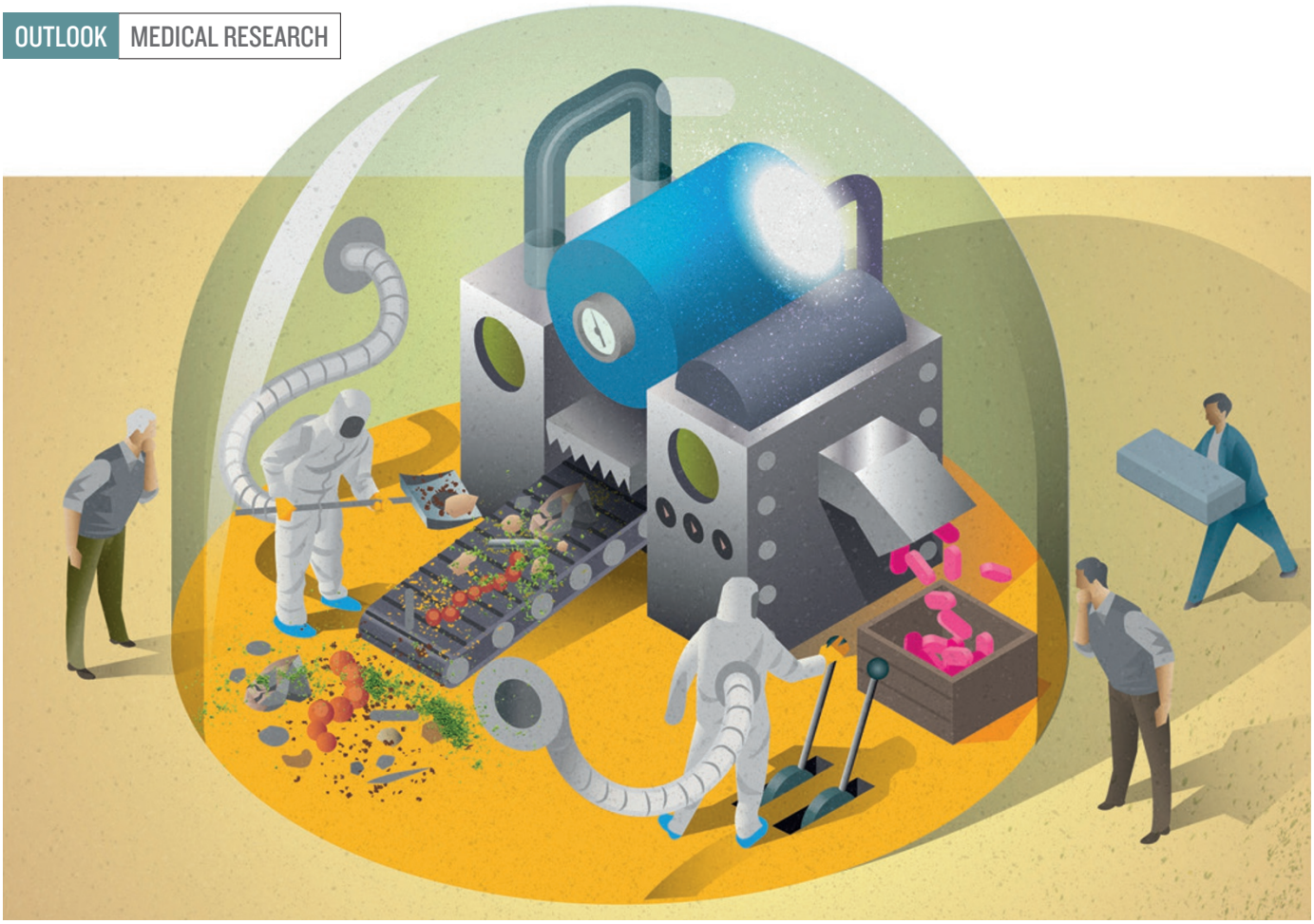


NILS-PETTER EKWALL

MOLECULAR BIOLOGY

Remove, reuse, recycle

Waste removal is not usually described as sexy, but the once-neglected field of autophagy — which plays a part in cancer and other diseases — is a hot topic in biomedical research.

BY MICHAEL EISENSTEIN

When Ana María Cuervo began researching her thesis in autophagy — a cellular recycling mechanism — little did she know that two decades later she would be working in one of the most dynamic fields of medical research. Randy Schekman, winner of last year's Nobel Prize in Physiology or Medicine, even chose to talk about autophagy in his opening address to the 37 laureates and 600 young scientists at this year's meeting instead of cellular trafficking — his prizewinning work. Cuervo is accustomed to this rise in interest. "I did my thesis on autophagy in the early 1990s when autophagy wasn't cool," says Cuervo, who is now co-director of the Einstein Institute for Aging Research at the Albert Einstein College of Medicine in New York City. "When I finished, everybody told me to change fields because autophagy was a dead end," she confesses. Studies have proved this prediction to be spectacularly wrong.

Autophagy was once considered to be little more than a cellular recycling bin — a process by which cells break down unwanted biomolecules into raw materials. But more recent research has revealed that autophagy is, in fact, a nexus for the cellular stress response and a failure point for many diseases. In the past ten years, researchers have made connections between autophagy and the immune response, cancer, neurodegeneration and ageing, says Daniel Klionsky of the University of Michigan in the United States. "The field just exploded."

A PROMOTION FROM HOUSEKEEPING

There are different types of autophagy, but the best-understood pathway is known as 'macroautophagy' — a bulk mechanism for gathering up and degrading proteins, organelles and other cellular materials. The process begins with the formation of a double-membrane structure known as a phagophore, which elongates and engulfs nearby cellular components (see 'Eating up the cell').

Autophagy was discovered in the 1960s, based on microscopic observations of selective degradation of cellular material within the lysosome (see 'A history of autophagy'). Over time, scientists accumulated evidence that this process helped cells to deal with nutrient-poor conditions, to eliminate excess proteins and even to remove entire mitochondria — the cell's metabolic power plants. However, most functions seemed to fall under the umbrella of basic maintenance, and autophagy research remained a niche field.

The turning point that showed autophagy was not simply cellular housekeeping came in the mid-1990s, when a number of proteins (now known as Atg proteins) that collectively mediate the formation and maturation of the phagophore were reported. Since then it has become clear that the Atg machinery intersects with physiological processes underlying an array of disorders, but scientists are still struggling to figure out the conditions that autophagy prevents or promotes.

CANCER CONTROVERSY

Autophagy seems to provide a crucial bulwark against genetic and biochemical damage — for example, by eliminating damaged mitochondria that would otherwise leak toxic molecules into the cell. As such, it is perhaps unsurprising that cancer was the first disease to be linked with autophagy. However, current evidence suggests that autophagy can act as both an enabler of and a protector against tumour growth, creating some debate in the field.

In 1999, Beth Levine and her colleagues at Columbia University, New York, showed that a protein called beclin-1 suppresses tumour activity in humans and promotes early formation of the phagophore[1]. The group also found that several cellular pathways that drive tumour growth inhibit autophagy, either by preventing activation of beclin-1 or by interfering with other Atg proteins. Levine is waiting for proof before declaring that autophagy failure itself drives tumour growth, but she believes it makes for a compelling hypothesis. "The general view is that autophagy plays a protective role against the development of cancer," she says.

However, some scientists believe that autophagy can also help advanced tumours to thrive by allowing cancerous cells to cope with the stress associated with competing for limited nutrients and oxygen, not to mention the toxicity caused by radiation or chemotherapy. Autophagy inhibitors could, therefore, render established cancers more vulnerable to treatment, says oncologist Ravi Amaravadi at the University of Pennsylvania in Philadelphia. "The overarching theme is that autophagy is an adaptive stress response that protects the cancer cell in advanced disease," he says.

KEEPING A CLEAR MIND

But it is not only cancer that is linked to the failure of autophagy — it also seems to play a key part in neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases. These conditions are characterized by the formation of dense protein aggregates, which point to some sort of failure in cellular housekeeping, but disruptions vary considerably between the conditions.

For example, neurons in Alzheimer's patients exhibit increased numbers of autophagosomes, the membranes that enclose the cell components before they are broken down, yet they can no longer fuse effectively with the lysosome.

Although the roots of Alzheimer's pathology remain unclear, with toxicity linked to accumulation of two proteins called tau and amyloid-β (Aβ), autophagic failure could provide a reasonable explanation for either pathway. "At late stages of disease you get what looks like an autophagy blockade that might compromise the whole process," says neuroscientist David Rubinsztein at the University of Cambridge, UK. "That's going to affect not only tau and Aβ clearance but also removal of damaged mitochondria and other processes."

By contrast, some forms of Parkinson's are associated with disruptions in a parallel autophagy pathway called chaperone-mediated autophagy in which specific proteins are delivered directly to the lysosome for degradation by means of a protein called LAMP2A without involvement of the autophagosome.

One of the proteins normally removed by this process is α-synuclein, the plaque-forming protein associated with Parkinson's. Mutant forms of the protein or an excessive production of it can gum up the system and cause a gradual but steady decline in neuronal health. "Chaperone-mediated autophagy cannot remove the molecules at the normal rate, and the protein begins to accumulate," says Cuervo. The normal autophagic process can compensate to a certain extent. However, as Rubinsztein and others have observed, α-synuclein can also exacerbate the condition.

CONSTRUCTIVE FEEDBACK

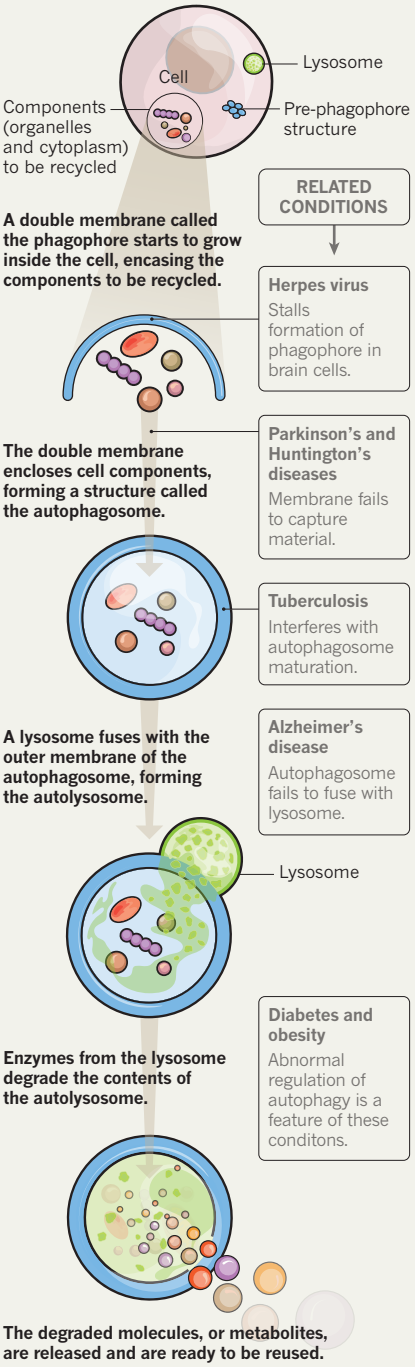
The impact of autophagy goes beyond the confines of an individual cell — this process is also used to regulate metabolic function throughout the entire body. Cuervo and her team recently found[2] that the liver helps to manage metabolism by using chaperone-mediated autophagy to selectively destroy the enzymes that convert sugar into energy. This is crucial, says Cuervo, because otherwise the liver becomes a "selfish organ" that uses all the glucose for itself at the expense of other tissues. Along with her colleague Rajat Singh, she has also found[3] that nutrient-sensing functions mediated by autophagy help the brain to convey that it is time to eat by switching on appetite signals and switching off those that indicate satiety.

Elements of the autophagy machinery also act as a line of defence against viruses and bacteria by diverting would-be cell hijackers to the lysosome for destruction. Microbiologist Vojo Deretic at the University of New Mexico in Albuquerque hypothesizes that the autophagy machinery may have served as a primordial form of immunity in early evolutionary history, by helping the body to distinguish between molecular signatures that represent foreign threats and those that are indicators of 'self' and should be ignored.

Many pathogens have evolved strategies that can sabotage autophagy, which Deretic first encountered while attempting to understand how *Mycobacterium tuberculosis* lives inside immune cells. He found[4] that the bacteria were escaping destruction by selectively attacking a molecule that would otherwise transport them to the lysosome. Likewise, Levine has observed[5] that the herpes virus thwarts autophagy to survive within neurons. "It has a protein that binds to the beclin-1 protein and blocks its function," she says. "This is not necessary for viral replication *in vitro* but is essential for replication in neurons, and this meant that viral evasion of autophagy was necessary for disease."

EATING UP THE CELL

Autophagy is part of a cell's normal function, removing proteins, damaged organelles and other unwanted material. Failure of the system is implicated in a number of conditions and ageing.



With so many crucial processes seemingly converging on a single cellular pathway, the expectation is that failures in autophagy have far-reaching consequences throughout the body. The evidence now strongly suggests that ageing is associated with a decline in autophagy, and some researchers are intrigued by the striking overlap among conditions that are associated with both ageing and autophagy, such as

diabetes, cancer and neurodegenerative disease. Cuervo and her colleagues have found evidence that chaperone-mediated autophagy might be an important factor in healthy human ageing. For instance, her team learned that the receptor in this pathway (LAMP2A) normally decreases with age, and therefore reduces the cell's ability to degrade proteins, which Cuervo believes could increase the risk of metabolic diseases. This initiates a vicious circle, whereby failure to control enzymes that break down sugar and fat leads to their steady accumulation in the body which, in turn, further suppresses autophagy. The inability of the cell to maintain its internal environment could be linked to other ageing-associated disorders, too. "It's like my mother used to say: in a clean house, everything works better," says Cuervo.

Conversely, other tricks to boost longevity seem to demand healthy autophagic function. For example, caloric restriction — in which subjects greatly reduce their food consumption without crossing the line into malnutrition — has been strongly linked with increased lifespan in many animal models. These same physiological conditions also stimulate autophagy, offering tantalizing evidence that these two processes — autophagy and the longevity gains associated with restricted caloric intake — may be linked. Research from Levine's group has also shown that exercise can stimulate autophagy, and she speculates that our well-fed and sedentary contemporary lifestyles may suppress our capacity to maintain the high level of autophagy that helped to keep our ancestors healthy.

HUNGRY FOR NEW THERAPEUTICS

The potential link between increased autophagy and better health could be good news from a therapeutic perspective. The Levine group has developed a promising molecule that can stimulate autophagy, protecting mice against otherwise-lethal viral infections and blocking the accumulation of proteins associated with neurodegenerative disease in cultured cells.

Rubinsztein's team has obtained promising preliminary results in mice with an autophagy-stimulating drug called rilmenidine, which has already been approved for treating high blood pressure in the United States and Europe. The drug is being tested in an ongoing clinical trial for safety in patients with Huntington's disease, and Rubinsztein hopes to move towards efficacy trials in patients with early stage neurodegenerative disease — an area where many clinical researchers see the greatest promise in autophagy-targeting therapeutics.

Several pharmaceutical companies, including Novartis, Pfizer and Millennium, are testing the waters, primarily focusing on inhibiting autophagy to make cancers more susceptible to chemotherapy treatment. In August 2014, Amaravadi and his colleagues published half a dozen phase I trials in which they paired different cancer treatments with hydroxychloroquine, an antimalarial drug that also impairs lysosomal degradation. Although the results were ambiguous in terms of efficacy, the safety profile seems favourable. Amaravadi also reported evidence of stalled autophagy in blood cells and tumour tissue from patients treated with the highest doses of hydroxychloroquine, suggesting that this drug or a related compound might be able to thwart a mechanism by which cancer eludes destruction.

Given the ambiguous role of autophagy in helping or hindering cancer, experts have expressed concern that the genetic heterogeneity found within a typical tumour could make cancer too challenging a target for such a broad therapeutic approach. "I'm not optimistic that this pro-survival function of autophagy is going to be a good therapeutic in all or even most cancers," says Levine. At least one study[6] suggests that inhibiting autophagy might instead provoke more aggressive tumour growth, although another study[7] has contested those findings. For now, this remains a topic of considerable debate, and Amaravadi hopes to gain deeper insights in an upcoming phase II trial in patients with pancreatic cancer. "This is very important because it's randomized, so if there's a signal we'll know that it's due to the hydroxychloroquine," he says.

From a therapeutic perspective, hitting the wrong target could have dire consequences. "If you're having problems with autophagosome clearance, as has been shown with Alzheimer's, then a drug that promotes autophagosome formation will just create more vesicles that aren't going anywhere and make a bad traffic jam worse," says Cuervo. Furthermore, some viruses actually make use of the autophagy machinery to assist in replication, so the same drug that thwarts, say, herpes might encourage poliovirus proliferation and release.

Additionally, many of the drugs being tested affect autophagy either incidentally or in conjunction with other cellular pathways, making it harder to determine whether autophagy is the culprit or the cure for a given condition.

BACK TO BASICS

Deretic has obtained promising early data from a compound that may help to contain the proliferation of HIV by means of autophagy, but wants to get a better insight into how the molecule works before getting too excited. "We have to be very careful about how we interpret the data and what we expect to see before we even start the experiment," he says. "Is it an inducer or an inhibitor, and is it driving the whole process or just half of it? A lot of screening data stop short of answering these questions."

These questions become even harder to answer in the clinical setting, where researchers often rely on proxy indicators to glean static snapshots of a highly dynamic process. Klionsky has worked with many of the field's top researchers to devise best practices for studying autophagy, but it can still be fiendishly difficult to determine how a given experimental manipulation is altering the process — especially when one is targeting cells deep within the brain or liver.

For this reason, some of the most important near-term studies in autophagy will be basic research efforts that monitor the nuts and bolts of the process. "We need to understand how autophagosomes are built, what regulates the way they form and what regulates their itinerary within the cell and fusion with lysosomes," says Rubinsztein. "Having that toolkit expanded will give us more potential insights into links with different types of disease." ■

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BACK IN TIME

A history of autophagy

The story of autophagy begins with Belgian cell biologist Christian de Duve, who shared the Nobel prize in 1974 for his exploration of the structural and functional organization of the cell. De Duve discovered the lysosome, an acidic membrane within the cell that is loaded with enzymes that can digest biomolecules. In the 1960s, scientists learned that proteins and structures called organelles within cells were being scooped up from the cytoplasm and delivered to the lysosome for destruction and recycling. De Duve coined the term 'autophagy' to describe such cellular self-cannibalization.

Early studies linked autophagy to the body's ability to sense nutrients, suggesting that the process enables cells to obtain raw materials during starvation. Once this model was established, interest in the subject waned. Things changed in the mid-1990s when researchers began to untangle the mechanisms that drive autophagy. From studies in simple organisms, such as yeast, scientists built genetic and functional maps of the machinery used in autophagy. It became clear that autophagy was conserved throughout evolution and served a more crucial purpose than just providing emergency rations to cells. **M.E.**

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