Recycling precious materials is nothing new – cells have been doing it for ages.

AUTOPHAGY, AGAIN AND AGAIN

By Sarah Williams

Recycling glass, paper, and plastic may be a purely human endeavour, but cells have perfected their own form of recycling, called autophagy. Once dubbed a "garbage pathway", autophagy is now turning out to be far more complex – and have further-reaching impacts on health and disease – than scientists ever guessed.

You are made of stardust. The atoms in your big toe, your brain, and the overwhelming majority of everything in between predate you by billions of years, hailing from the Big Bang and from distant, ancient stars. These atoms have been recycled again and again – making their way through time until they ended up in your body.

Inside all living cells, these cosmically born atoms make up the building blocks of proteins, fats, sugars, and DNA. When they have served their purpose, these macromolecules are recycled. Again and again, molecules are broken down into their components to build entirely new structures.

Christian de Duve coined the term autophagy – literally "self-eating" – more than 50 years ago to describe the process in which eukaryotic cells gather up molecules and whole organelles from inside the cell to be reduced to their building blocks. Today, it is clear that autophagy is vital to the normal functioning of cells. The recycling process, among other things, helps cells balance sources of energy when they are under stress. For example, mitochondria – which rely on oxygen to generate energy – undergo autophagy when there is a shortage of oxygen.

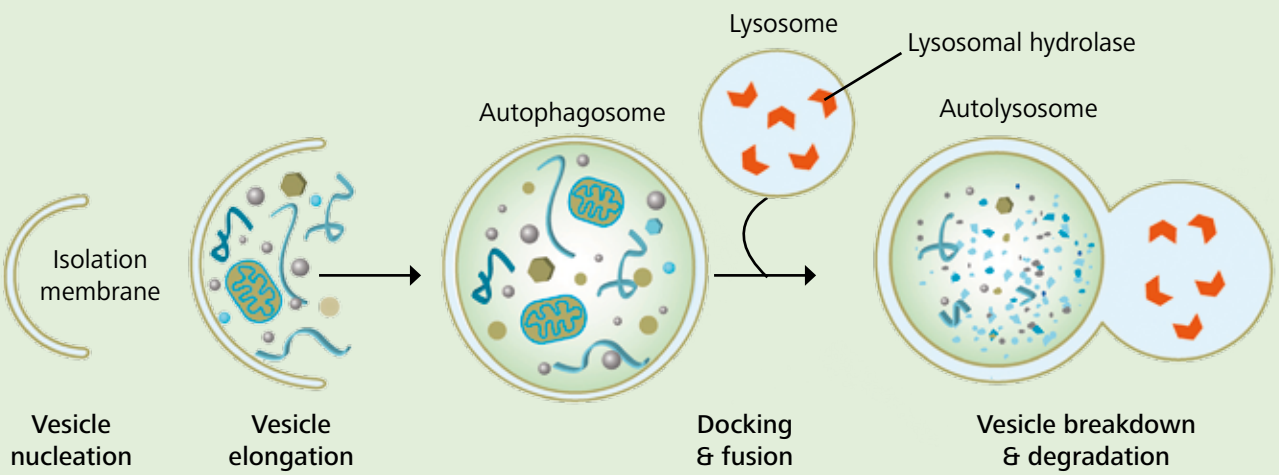
For nearly three decades, though, autophagy was shrugged off as a "garbage pathway," and studied only tangentially. Most assumed that autophagy had little effect on how cells functioned, and technically, it was difficult to study autophagy – there were no methods to track the process other than watching it under a microscope, and no known autophagy genes. Instead, researchers focused their attention on ubiquitin-mediated protein degradation, which had clear implications for controlling levels of regulatory proteins in cells.

In the 1990s, though, a series of discoveries helped pique scientists' interest in autophagy. In the span of just a few years, Yoshinori Ohsumi, then at the University of Tokyo in Japan, developed an assay for measuring autophagy in yeast cells, and used the approach to discover 15 autophagy-related genes, the ATG genes. His group went on to uncover mammalian versions of the yeast genes, paving the way for researchers to start making links between autophagy and human health. When, in 1999, Beth Levine of the University of Texas Southwestern Medical Center in the USA reported that Beclin-1, a mammalian autophagy gene, could inhibit tumour formation – the first link between autophagy and disease – the field really took off. In 2016, Ohsumi won the Nobel Prize for his pioneering work.

While basic questions remain about autophagy and its regulation, scientists have identified a number of pathways that intersect with it and countless links between autophagy and human disease have been uncovered. →

Photo: Jan von Holleben

HOW MACROPHAGY WORKS



Macroautophagy was the first type of autophagy to be discovered as it can be observed under the microscope.

De Duve first described autophagy in liver cells. They use it as a way to get energy from unneeded molecules when levels of nutrients in the liver decrease – between meals, for instance. During the first eight hours of nutrient deprivation, the liver breaks down proteins, including many found in energy-generating mitochondria, which are themselves engulfed through autophagy. After eight hours of starvation, the liver's autophagy begins to focus on lipids, engulfing lipid droplets – stores of triglycerides and cholesterols. Immediately after a meal – when blood sugar levels rise again – autophagy is curbed.

Research since de Duve's time has revealed reasons other than starvation that cells throughout the body turn on autophagy. Proteins, organelles, or DNA molecules that are damaged can all spur the process. Misfolded proteins, in particular, might disrupt a cell's normal functioning if allowed to accumulate. Infection, oxidative stress, or a lack of oxygen can also trigger autophagy, using the programme as a way both to destroy invading bacteria or viruses and conserve energy to avoid cell death.

"Autophagy is often a survival mechanism," says Ioannis Nezis of the University of Warwick in the UK. "A cell is eating [parts of] itself as a last effort to survive under stress." In other cases, a cell may need to destroy proteins or other molecules to massively shift its shape or function, as happens often during development, stem cell differentiation, and immune responses. And even in the absence of these triggers, it seems the cell is constantly engaged in low levels of "quality control" autophagy to help encourage the turn-over of long-lived proteins and organelles which may have accumulated damage over time.

Not all autophagy, though, looks the same; three different forms have been described. All three forms of autophagy involve moving cellular components to the lysosome, a compartment in the cell that is optimized for digesting molecules. With a low pH – like the stomach – this membrane-bound section of the cell contains enzymes that can break apart proteins, carbohydrates, lipids, and nucleic acids. But there are large differences between types of autophagy when it comes to how materials get to and into the lysosome and how much is moved in one go.

When most scientists talk about autophagy, they really mean macroautophagy – it is the best understood of the three varieties, and the longest studied. It is also the kind that is easiest to watch under a microscope: a double membrane begins to assemble, first forming a shape like a catcher's mitt that can grab nearby material floating in the cell's soupy cytoplasm. As the membrane – called the phagophore or isolation membrane – continues to grow, it closes around the material to be broken down, forming a sphere known as an autophagosome. The autophagosome – a floating recycling bin of sorts – moves to the

lysosome with its cargo, which can include multiple different types of molecules in one load. The autophagosome then fuses with the lysosome. The resulting vesicle is called an autolysosome; everything in it is broken down by the lysosomal hydrolase.

For a number of years, scientists assumed that macroautophagy was always non-selective; the phagophore seemed to just grab whatever was nearby in its attempts to salvage energy. But we now know that the phagophore can also enclose materials that are specifically chosen to be recycled. For instance, when a cell's RNA damage repair response senses a double strand break, a selective form of macroautophagy is activated to destroy the damaged DNA. In other cases, the cell uses this type of specific macroautophagy to recycle entire organelles, and each subtype has its own name: mitophagy to digest mitochondria and ribophagy to digest ribosomes, for instance.

In the mid-1990s, just as Ohsumi was identifying the ATG genes required for macroautophagy in yeast, scientists including Ana Maria Cuervo from the Albert Einstein College of Medicine in New York City, USA, also revealed an entirely new type of autophagy, called chaperone-mediated autophagy (CMA) specific to mammals. CMA transports proteins that have a sequence of five specific amino acids that are kept hidden within the proteins' structures at most times. The sequence can be exposed, however, when a protein is misfolded due to errors in the folding process or unfolded due to damage – which in either case is potentially dangerous to the body. When this happens, the cytosolic heat shock protein Hsc70 binds the exposed sequence. Together, Hsc70 and the target protein move to the lysosome, where Hsc70 helps the complex to dock at the lysosomal membrane. There, a separate molecule, LAMP-2A, which extends through the lysosomal membrane, pulls the tagged protein directly into the interior of the lysosome without the help of a vesicle.

Like macroautophagy, CMA was first discovered in liver cells, the champions of recycling in our bodies. But CMA has more recently been shown to be critical to clearing damaged proteins from cells in other parts of the body, too. In the brain, where new cells are rarely formed, removing damaged proteins from existing cells before they can trigger cell death is particularly vital. As opposed to macroautophagy, in which functioning proteins may be non-specifically swept into an autophagosome, CMA helps ensure that only damaged proteins, with the five-amino-acid sequence exposed, are moved to the lysosome.

There is a third type of autophagy, called microautophagy, which researchers are still working to understand. Materials can be moved to the lysosome using Hsc70, as in CMA, or through other means, and the process can be either specific or non-specific, like macroautophagy. But once the molecules are near the lysosome, they are not moved across the lysosome membrane by LAMP-21 as happens in CMA. Instead, they are internalized when the lysosomal membrane blebs inward, forming a vesicle. In general, these vesicles – which form at the surface of the lysosome once materials are already in the vicinity – are smaller than the vesicles which form in the cytosol to move materials to the lysosome in macroautophagy. So far, most of what is known about microautophagy comes from studies in yeast. The three types of autophagy operate using their own machinery, but are far from independent, researchers are discovering.

In some cases, when one form is blocked, the others might increase their activity. Other times, the same mechanisms – cellular damage or starvation, for examples – boost levels of all the types of autophagy at once.

To study autophagy, researchers have developed systems that let them track where and when autophagy is occurring by watching the programmes in action in isolated cells and tissue cultures. The methods are letting them move past basic observations on how and when autophagy occurs to ask more complex questions about how it is regulated. "Both too much and too little autophagy is a problem," says Daniel Klionsky of the University of Michigan in Ann Arbour, USA, who studies →

When most scientists talk about autophagy, they really mean macroautophagy – it is the best understood of the three varieties, and the longest studied. It is also the kind that is easiest to watch under a microscope.

macroautophagy in yeast. "And there's a huge range of mechanisms that cells are using to regulate all the genes and proteins involved in the process."

Klionsky has been on the hunt for regulatory genes that impact the expression of the known ATG genes. To study the potential effects of genes identified in screens, he often turns to a fusion protein that is used widely in the field: a combination of green fluorescent protein (GFP) and Atg8, a protein that remains associated with the autophagosome from its formation until its fusion with the lysosome. Scientists can see fluorescent dots when Atg8 is produced and integrated into an autophagosome.

In one recent *Current Biology* paper, Klionsky's team uncovered the role of a gene known as Pho23 in autophagy using this approach in yeast. They found that Pho23 controls the expression of the autophagy gene ATG9, which, in turn, regulates the frequency of autophagosome formation.

Compared to macro- and microautophagy, studying CMA is complicated by the fact that yeast and *Drosophila* do not have a CMA pathway; it is specific to mammals. But in mammalian cells, Cuervo's team has developed their own approach. They couple a fluorescent protein and the five-amino-acid sequence that targets proteins for CMA. When the proteins are added to rat or mouse cells, they move toward the lysosome and, depending on levels of LAMP-2A, are eventually pulled inside the organelle and degraded. In one experiment, Cuervo used this fluorescence approach on a variety of cell types, including neuroblastoma, hepatoma, and breast adenocarcinoma, to compare their rates of CMA when the cells were removed from protein-rich serum, a stressful change that often triggers autophagy. The hepatoma and breast adenocarcinoma cells, the researchers found, had higher basal rates of autophagy but upregulated the process less than other cells when starved of serum. The implications of this on cancer treatment are not yet clear.

Cuervo and others have also discovered that as humans age, levels of autophagy in their cells go down. In flies and worms, mutations that dampen or knock out autophagy decrease lifespan. Proteins involved in various steps of all three types of autophagy seem to work less well – or have lower levels – in older animals. Cuervo has shown that for CMA this can be attributed to a particular receptor on the surface of lysosomes that becomes less stable with age. Reduced autophagy could thus be one reason why the risk of some diseases increases with age.

New research about autophagy, therefore, has the potential to offer valuable insights into human health – and maybe even lead to new drugs. If scientists can learn how to turn up autophagy in specific cells and tissues, they may be able to stop the accumulation of damaged proteins and help cells metabolize correctly. The first could be a boon for treating Alzheimer's, Parkinson's, and Huntington's diseases, the latter could be useful for treating metabolic disease including diabetes. The accumulation of damage in the mitochondria of cells has been implicated in insulin resistance, and some researchers have suggested that ramping up autophagy to remove defective mitochondria could help treat or prevent diabetes. In addition, since Levine's first paper on Beclin1, scientists have uncovered a complex relationship between autophagy and cancer, with the early formation of tumours often linked to a suppression of autophagy, and more advanced tumours relying on high rates of autophagy to survive stress. To date, no drugs on the market have been developed specifically to target autophagy, but some previously approved ones – including metformin and lithium – interact with proteins involved in autophagy and have been shown to change levels of autophagy in cells.

But with hypotheses on the roles of autophagy in human health and disease, scientists keep hitting the same brick wall. There is currently no way to track autophagy in living animals, let alone people. "This is one of the biggest challenges right now," says Klionsky. "If you want to modulate autophagy for therapeutic purposes in humans, you want to do know if you're actually changing the process. Did your treatment actually do what you wanted?"

As researchers continue to study the basics of autophagy, they are continually reminded that the process is more than just destruction. After materials are broken down in the lysosome, molecules like amino acids and nucleic acids are transported back into the cytoplasm, ready to build new structures. "The beauty of it is that you both destroy unwanted things and use the pieces of what you're breaking down to synthesize new things," says Cuervo.