How do cells dispose of their trash?

Cells are biological wonders, akin to a bustling city on a molecular scale capable of metabolic reactions synthesising a vast array of products. As a result, plenty of ‘trash’ is produced. This can be defined as either the by-products of cellular respiration - such as water, carbon dioxide and lactic acid in human muscle cells\(^1\) - or proteins which have misfolded and have thus lost their native function as a result of failures during the synthesis of the polypeptide chain, genomic mutations or a diverse range of stress conditions\(^2\). In this essay the latter definition of trash is used, referring to potentially toxic protein species and damaged organelles. Much like how cities will grind to a halt without the work of rubbish collectors and waste disposal facilities, in order to prevent the accumulation of protein aggregates and possible damage to the cell’s molecular machinery, cells have several mechanisms\(^3\) to dispose of cellular trash. Describing these mechanisms is the main focus of this essay.

There are two main proteolytic pathways\(^2\) to dispose of the abovementioned trash: the first is the ubiquitin-proteasome system (UPS) which deals primarily with individual proteins. Within the UPS, misfolded proteins that cannot be refolded into their functional state are labelled using an ubiquitin tag. This is recognised by the proteasome, a sophisticated protease machine consisting of two subcomplexes: the regulatory particle (RP) which recognises the ubiquitin tag and controls the movement of proteins into the catalytic core particle (CP), which breaks down the protein into amino acids. Beyond waste disposal, the UPS also plays a significant role in regulating several cell processes from the cell cycle to immune responses\(^4\), as a result of the controlled degradation of particular signalling molecules.

The second system is the autophagic lysosomal system, which unlike the UPS breaks down larger components of the cell and is not limited to proteolysis. Larger intracellular dysfunctional proteins and senescent organelles in the cytoplasm are engulfed by a vesicle and digested by lysosomal enzymes, thus producing molecules that can be reused. Autophagy can selectively remove damaged organelles and protein aggregates, though when the cell is starved, components in the cytoplasm are digested randomly in order to release enough metabolites to synthesise essential molecules.

How trash is identified for each pathway

Before one can describe in more detail about the individual pathways, we must look at how cells identify waste. Through a series of studies conducted by Ciechanover, Hershko and Rose from the late 1970s to the early 1980s using a rabbit reticulocyte lysate system\(^4\) (the contents from the lysis of immature red blood cells)\(^5\),\(^6\) as their model for waste products, they discovered that cells are able to regulate the breakdown of proteins with surprisingly great specificity using the ubiquitin-tagging pathway. The importance of this discovery was recognised with the awarding of the 2004 Nobel Prize in Chemistry to them\(^3\). The ubiquitin-tagging pathway involves a cascade of three enzyme species: E1, E2 and E3. The E1(ubiquitin-activating) enzyme activates the ubiquitin, a highly-conserved 76 amino acid-long protein, by forming a thioester bond between the C-terminal glycine residue of ubiquitin and the cysteine residue in the active site of the enzyme. The ubiquitin is then bound to the cysteine residue\(^6\) of E2 (ubiquitin conjugating), before E3 (ubiquitin ligating) catalyses the formation of an isopeptide bond between the C-terminal of ubiquitin and a lysine residue of the substrate\(^7\). This cycle can be repeated multiple times resulting in a polyubiquitin chain on the substrate that can be detected\(^8\).
In order to differentiate between proteins for the UPS or autophagic lysosomal system, cells can organize dysfunctional proteins into separated quality control compartments within the cytosol. The insoluble protein deposit (IPOD) stores insoluble aggregates made of non-ubiquitinated misfolded proteins. This is subsequently covered with ubiquitin and is then recognized by an autophagy receptor which can bind to the ubiquitin chains and thus allow the aggregate to be degraded via selective autophagy. The second compartment is known as juxtanuclear quality control (JUNQ), containing ubiquitinated soluble misfolded proteins that are either refolded by molecular chaperones or are degraded by the UPS. Interestingly, differences in the ubiquitin linkages can direct a substrate towards a specific pathway. This is because ubiquitin has seven lysine residues (K6, K11, K27, K29, K33, K48 and K63), each of which can undergo further ubiquitination to form a specific polyubiquitin chain. These different chain linkages can convey different information - proteins with K63 chains are targeted for autophagic degradation, as stated above, whereas those with K48 chains will undergo proteasomal degradation. The different chains are due to the actions of certain E3 enzymes with a specific E2 enzyme, meaning that it is possible for some E3 enzymes to have the dual-function of directing a substrate to either pathway according to specific conditions.

The structure and function of the proteasome

The next stage of the UPS is the proteasome as aforementioned. The overall structure of the 20S proteasome, a CP, consists of a cylindrical complex of four stacked rings surrounding a pore, as shown in Figure 1. Each ring is made of seven α or β subunits: the two inner β-rings contain the active sites of the proteolytic enzymes and are kept within the pore, whilst the two outer α-rings are almost completely closed, in order to prevent access to the proteolytic active sites which could degrade proteins not intended for the UPS. The 19S proteasome is an example of an RP – it can bind to either one or both ends of the 20S proteasome, creating the active proteasome complex known as a 26S proteasome. The 19S RP can recognize the polyubiquitin chains decorating the substrate proteins and thus remove the chain, entrap the substrate, unfold it and feed it into the 20S pore. The substrate is then progressively degraded into oligopeptides with a length of three to fifteen amino acid residues, before being broken down further into single amino acids. The 11S proteasome is another RP which produces small peptides that are presented by MHC molecules, thus allowing the recognition of foreign substances. The 11S proteasome complex is thought to work cooperatively with the 26S proteasome, in that it further degrades polypeptides produced from the latter, presenting an interesting role in how proteasome complexes work together.

Although the proteasome complex is highly conserved among organisms due to its fundamental role in cells, there are differences. For instance, it has been found that cells are able to assemble different versions of proteasomes which in turn can degrade proteins slightly differently. Both yeast and human cells can overcome a lack of the α3 subunit by placing an α4 subunit in the position previously occupied by the α3 subunit, thus forming an α4-α4 proteasome.
alternative type of proteasome seems to deal with stresses such as toxic heavy metals more effectively, though they also appear to be assembled at a greater rate in certain cancers, suggesting a possible role in the survival and progression of these cancers\textsuperscript{17}. This could be due to the changes made in the $\alpha$-ring, which would affect the regulation of substrates entering the CP\textsuperscript{16}. A change in how specific proteins are degraded may contribute to cancer\textsuperscript{16}, perhaps by the dysregulation of the cell cycle or gene expression; therefore it may be possible to target these proteasomes as a cancer treatment\textsuperscript{17}.

The autophagic lysosomal system

Similar to the UPS, this system is also crucial for the removal of cellular waste. It involves the lysosome, a membrane-bound organelle that acts as a dismantling and recycling facility, filled with about fifty enzymes\textsuperscript{18} that can degrade all types of biological molecules. The interior of a lysosome is at an acidic pH of less than 5.0\textsuperscript{18}, lower than that of the cytosol at around pH 7.2\textsuperscript{19} – it acts as a protective mechanism, as this ensures that should the enzymes be released, they will be far less active at a different pH in order to prevent them acting upon the contents of the cytosol. The acidic conditions are maintained by proton pumps transferring hydrogen ions from the cytosol and into the lysosome interior\textsuperscript{18}.

The formation of a lysosome starts with the transportation of the lysosomal enzymes from the rough endoplasmic reticulum via the mannose-6-phosphate (M6P) recognition pathway\textsuperscript{14}. In the cis-Golgi apparatus, enzymes intended for lysosomes are first tagged with M6P on an asparagine residue\textsuperscript{20}. The M6P-enzyme complex then travels along the trans-Golgi apparatus towards M6P receptors, which recognise the enzyme and load the complex and receptor still bound to it onto a transport vesicle. The transport vesicle then travels to a late endosome, a membrane organelle derived from the endocytosis of material into the cell\textsuperscript{21}. The lysosomal enzymes are deposited inside it and due to its relatively low pH of around 6.0\textsuperscript{14}, the receptors dissociate and are transported back to the trans-Golgi apparatus where they can be reused. The late endosome meanwhile matures into a lysosome as it now contains a full set of lysosomal enzymes\textsuperscript{19}.

There are three main pathways by which material can be degraded by lysosomes\textsuperscript{18}. The first of which is the degradation of material internalized via endocytosis, where the plasma membrane invaginates and takes in external material in a vesicle. This fuses with an early endosome which can mature into a late endosome\textsuperscript{19}. Similar to the first method, the second pathway is via phagocytosis, wherein phagocytes engulf and surround cellular debris and antigens, forming a phagosome which can fuse with a lysosome\textsuperscript{18}. The third method, autophagy, is presented in detail below:

As illustrated in Figure 2, the autophagy process begins with nucleation\textsuperscript{22}, or the formation of the isolation membrane. This is a small cisterna, discovered to predominately originate from the outer membrane of the mitochondria with some contribution from the endoplasmic reticulum\textsuperscript{23}, which then elongates in the second step and surrounds the contents of part of the cytoplasm to form an autophagosome\textsuperscript{22}. The third step is
maturation, when the autophagosome is completed and transported to the perinuclear space (the space between the outer and inner membranes of the nucleus)\textsuperscript{24}, as while autophagosomes can be formed randomly within the cytoplasm, late endosomes and lysosomes are found mainly in the perinuclear space\textsuperscript{22}. Interestingly, the more localised the lysosomes are within the perinuclear space, the greater the rate of autophagosomal fusion, indicating the necessary coordination of the transport of both the lysosome and autophagosome at the right time\textsuperscript{22}.

Here, fusion occurs, wherein the autophagosome docks and fuses with a lysosome, thus forming an autolysosome. Alternatively, the autophagosome can fuse first with a late endosome, forming an amphisome, before fusing with a lysosome\textsuperscript{22}. The exact mechanism for the actual fusion is still unclear, as it is difficult to distinguish between the movement of the lysosome and autophagosome, and the fusion itself\textsuperscript{22}.

Based on current knowledge of intracellular trafficking, it is possible to say that the fusion involves the following: Rab GTPases, which regulate membrane trafficking, are localised to the specific membranes and use membrane-tethering complexes which act as bridges, bringing the compartments closer together. The tethering proteins then help SNARE proteins (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) to drive the actual fusion of the phospholipid bilayers\textsuperscript{22}. Finally, the sequestered material is degraded within the autolysosome. Once the material has been digested, the resulting useful molecules are taken into the cytoplasm to be reused and the lysosome is reformed. Any residue left is either removed from the cell via exocytosis or sealed up in a pigment granule for the remaining life of the organism\textsuperscript{18}.

The role of autophagy in cells

Autophagy itself has been increasing in interest within scientific research\textsuperscript{23}, due to its links to immune and metabolic diseases. When cells are under stress, autophagosomes are formed indiscriminately within the cell and their rate of assembly increases, in order to recycle enough intracellular material for metabolic pathways. This mechanism was most likely first developed in unicellular organisms to ensure an available pool of metabolites and perhaps later as a primitive immune defence\textsuperscript{23}. As such, multicellular organisms have retained the system, and in mammals specifically autophagy is induced by starvation and physiological stimuli like hormones, growth factors and pathogen invasion\textsuperscript{23}.

Despite this, autophagy also occurs selectively at a basal level in order to remove dysfunctional proteins and organelles to prevent cell damage. Mitophagy or the autophagy of mitochondria is one of the best-studied pathways for selective autophagy\textsuperscript{25}. To summarise, PTEN-induced kinase 1 (PINK1) distinguishes between damaged and healthy mitochondria. PINK1 then recruits Parkin, an E3 enzyme, which ubiquitylates outer mitochondrial membrane proteins. The ubiquitin chains are thus recognised and direct the formation of an autophagosome around the mitochondria\textsuperscript{26}.

There have also been links found between disease and autophagy: for instance, in some cases autophagy may suppress tumorigenesis, as autophagy deficiency can lead to many events which contribute to cancer, such as oxidative stress and genome instability – however it is unclear if autophagy itself contributes to the suppression of cancer\textsuperscript{27}. Instead, there is evidence that autophagy promotes tumours, since cancer cells are often more reliant on autophagy than normal cells, most likely due to deficiencies in nutrients in the environment and the increased demands from unregulated proliferation\textsuperscript{27}. It has been found\textsuperscript{27} that, when \textit{Atg7} (one of several autophagy-related genes) is deleted in tumours of genetically engineered mice with a type of lung cancer, the
tumour suppressor p53 gene is activated, causing cell arrest then apoptosis. How autophagy deficiency activates p53 is not yet known, though the gene alone is activated by oxidative stress, induction of DNA damage and metabolic deficiency - thus perhaps holding a clue as to how autophagy deficiency is linked to p53. However, deleting p53 along with Atg7 has a similar result, indicating that while autophagy deficiency can activate p53 which in turn suppresses tumour growth, the deficiency itself limits tumorigenesis independent of p53. Autophagy inactivation could therefore be utilised therapeutically as a cancer treatment, though care must be taken that the inactivation is sufficiently selective so as to not cause any normal tissues to become autophagy deficient and possibly suffer deleterious consequences.

The importance of waste disposal in cells cannot be demonstrated more clearly than in the diseases that develop with its misregulation. Misfolded proteins can clump together into aggregates which have been linked to neurodegenerative diseases. There are nearly fifty lysosomal storage diseases, all associated with a deficiency in a lysosomal enzyme or an error in the destination label of the enzyme, leading to the accumulation of potentially toxic material in the cells. Increased understanding of how cells dispose of their trash could therefore lead to new approaches in the treatment of diseases, creating hope from the waste disposal facilities of the cell.

**Bibliography**


