# Autophagy: where next?

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Autophagy refers to a set of diverse processes whereby intracytoplasmic material is delivered to lysosomes. Macroautophagy—hereafter referred to as autophagy—is characterized by the formation of double-membrane vesicles around portions of cytoplasm to form autophagosomes. These eventually fuse with lysosomes, where their contents are degraded. Another form of autophagy, known as chaperone-mediated autophagy (CMA), occurs when a pentapeptide motif in a target protein is recognized by Hsc70, thus facilitating the delivery of the protein to LAMP2A on lysosomes. In CMA, LAMP2A functions as a receptor and pore for substrate uptake into lysosomes. Microautophagy, the least understood of these processes in mammalian cells, occurs when portions of the lysosomal membrane directly engulf nearby cytoplasm.

The discovery of autophagy genes (Atg) in yeast initiated an exciting era in research, as it helped ascribe biological effects to this process. The subsequent identification and functional validation of mammalian orthologues of these genes has led to the appreciation that autophagy is crucial for diverse physiological processes, including starvation responses, immune function, lipid metabolism, erythropoiesis, development and protection from cell death. Autophagy is also a key modulator of disease, from neurodegeneration (autophagy clears intracytoplasmic aggregate-prone proteins) to infectious diseases (various pathogens can be degraded by autophagy) and cancer (loss of autophagy might predispose to tumorigenesis and aneuploidy).

Another form of autophagy has been described recently that seems to be independent of Atg5, Atg7 and some other key components of the classical autophagy machinery (Nishida *et al*, 2009), and occurs when cells are exposed to etoposide. However, it is not known to what extent this form of autophagy contributes to protein catabolism under normal physiological situations, or during disease. Likewise, the extent to which CMA and microautophagy contribute to protein breakdown or the clearance of specific substrates is unclear under many physiological conditions.

Despite the progress that has been made in understanding autophagy, many issues remain unresolved. The sources of membrane that give rise to autophagosomes are still mysterious, although the endoplasmic reticulum could be involved (Axe *et al*, 2008). At the molecular level, it is still unclear how many components of the autophagic machinery cooperate to allow the entire process to occur. Likewise, there is only limited understanding of how many of the major signals that regulate autophagy influence the autophagy machinery, although important steps have been made by showing that mTOR inhibits autophagy through the phosphorylation of Atg1 orthologues in mammalian cells (Ganley *et al*, 2009; Hosokawa *et al*, 2009; Jung *et al*, 2009).

Autophagy was long considered to be a nonselective bulk protein degradation system. However, recent work has suggested that there might be selectivity, at least for certain substrates. This could occur through the binding of ubiquitinated substrates to the ubiquitin binding protein p62, which might target them to autophagosomes by binding to one of their key components, LC3. Earlier data strongly suggested that such a process might occur, and further support has been provided recently in the context of bacterial autophagy (Zheng *et al*, 2009). It will be interesting to identify other substrates that are dependent on selective autophagy to enhance their clearance—that is, for which a loss of the selective component, without changes in autophagy flux, retards clearance. Other adaptor proteins might also be involved in the delivery of specific substrates to the autophagy machinery independently of p62; for example, it has been shown recently that Atg32 targets mitochondria to the autophagy machinery in yeast (Okamoto *et al*, 2009; Kanki *et al*, 2009). Although no orthologues of Atg32 have been identified, it is tantalising to consider that there might be a functional equivalent in mammalian systems, given the importance of mitochondrial clearance in the regulation of cell homeostasis.

Considering the importance of autophagy in diverse physiological processes, there might be interesting areas of metabolic control that involve a co-regulation of autophagy and other pathways. There are precedents for this: Bcl2 and Bcl-XL are anti-apoptotic proteins that also inhibit autophagy. Atg genes could also have functions that are distinct from autophagy; for example, Atg5 has been implicated in cell death pathways (Luo & Rubinsztein, 2007).

Much of the research in autophagy has used knockout animals (or cells) to study how various processes are perturbed. Although this strategy has been invaluable, some issues are worth considering in the future. Some of the consequences of knocking out Atg genes might not be specific autophagy properties. Indeed, the impairment of autophagy ultimately hinders flux through the ubiquitin-proteasome system (UPS), the other major catabolic route in cells. It is thus possible that some of the effects of decreased autophagy are due to a secondary impairment of the UPS. In addition, autophagy knockdown is an extreme situation and might not be a reliable mimic of autophagy-deficient situations in physiological or pathological states, which are likely to be less severe. Furthermore, complete autophagy knockouts have extreme phenotypes that might mask more subtle, but physiologically relevant, phenomena. The use of Atg hypomorphs might reveal additional roles of this pathway. In this respect, it is unclear how changes in autophagic flux in the limits of normal physiological variation influence different processes.

One of the future challenges will be studying autophagy in whole organisms. This will not be easy, as the toolboxes for reliably inferring autophagic flux *in vivo* are limited, especially in relatively inaccessible tissues such as the brain. Although progress is being made by developing various autophagy assays for cell-based methods, further methodological development will be required to assess autophagic flux in living mammals.

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