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# The autophagosome is overrated!

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For some people, the appearance of autophagosomes by electron microscopy is the "gold standard" for demonstrating macroautophagy. I could quibble with this idea for various technical reasons. For example, just how many autophagosomes are needed per cell to truly indicate an increase in autophagy, and how often do people carry out morphometric analyses to quantify the volume of autophagosomes relative to the total cytoplasm? In how many cases have researchers monitored autophagic flux by electron microscopy alone? Furthermore, how many times have papers claimed to be looking at autophagosomes based on electron microscopy without any substantiation, such as the use of immunostaining with antibodies to detect Atg8/LC3? However, complaints such as these would miss the point. The autophagosome is certainly a striking structure with its double membrane and relatively large size; accordingly, it is relatively easy to detect, particularly when conditions are present that cause it to accumulate—in which case these compartments can become a substantial part of the entire cytoplasm. Let me also make it clear that I do not question the value of electron microscopy for the study of macroautophagy.

Nonetheless, when it comes right down to it, the autophagosome is not very exciting. After all, we are talking about a relatively terminal structure. On the one hand, by the time the autophagosome has formed, a large part of the process of macroautophagy is over, at least with regard to the known autophagy-related components. On the other hand, it would be a mistake to view the autophagosome as the "end" of macroautophagy. For example, the autophagosome needs to fuse with the lysosome/vacuole, the cargo (at least most of it) needs to be degraded, and the breakdown products need to be released back into the cytosol. Without these steps, macroautophagy has not only failed to achieve its goal (in the case of starvation-induced macroautophagy), but the accumulation of autophagosomes that are not efficiently cleared can be deleterious to cellular physiology. Thus, autophagosome formation is not an end unto itself. However, I would venture to say that the steps after autophagosome formation, while important, and perhaps even fascinating, in their own right, are not as critical as the sequestration step; fusion and degradation will normally take place once the autophagosome has formed, and these steps are not even unique to macroautophagy. With regard to the latter point, all transport processes that target vesicles to the lysosome/vacuole, including endocytosis and the vacuolar protein sorting pathway, use similar components to drive fusion of the vesicular intermediate with the degradative compartment. Furthermore, the steps of degradation and efflux, while being important, are generally less exciting (although there are certainly many questions that remain to be answered in this regard, including the identification of lysosomal/vacuolar lipases and nucleases, or the fate of degraded nucleic acids, lipids and carbohydrates that have not yet been revealed). In contrast, with sequestration we have many truly compelling questions such as the origin(s) of the sequestering membrane, the mechanism used to drive membrane curvature, or the various aspects of selective cargo recognition.

Therefore, I would argue that it is the process of sequestration that is at the heart of autophagy. Certainly this is a more encompassing view, one that is less macroautophagy-centric. I mean that microautophagy-like processes and chaperone-mediated autophagy do not involve the formation of autophagosomes, but they are still interesting. What these all have in common is a sequestration event. In the case of microautophagy-like degradation, the sequestration occurs at the lysosome/vacuole limiting membrane and involves a dynamic rearrangement—a protrusion, septation and/or invagination—of that otherwise placid organelle surface. In chaperone-mediated autophagy, the lysosome membrane may not appear to experience such dramatic changes, but it is still undergoing various types of lateral mobility involving lipid rafts, the movement of LAMP-2A and its assembly/disassembly into and out of oligomers. As with macroautophagy, it is the steps that drive these events of sequestration that are the most fascinating, not what happens after the sequestration has occurred.

This brings me to the final point of this article. Whereas the autophagosome is overrated, the phagophore is highly underrated. It seems I am always reading about autophagosomes, when the real focus should be on the phagophore. What a marvelous structure, so transient, so poorly defined. How does it expand? Does expansion involve vesicular fusion or lateral membrane flow from an existing organelle? If the former, are SNAREs involved? What dictates the extent of expansion? For nonspecific autophagy, expansion presumably relates to curvature; with selective types of autophagy the mechanism might involve recognition of the cargo, but there are limits to the size of Cvt vesicles and mitophagosomes. Also, how does the phagophore undergo a final scission or fusion step to become an autophagosome?



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To see if my concerns are warranted, and to back up my claim of bias with some data, I carried out a search in PubMed looking for papers with the word "autophagy" in the title, and the publication year of 2011 (I wanted a small sample size). I did this in December 2010 and got 46 hits (I do not mind pointing out that 31 of them were for papers being published in *Autophagy*). I then looked at the abstracts and found that 13 of them referred to autophagosomes, whereas none of them mentioned phagophores:

"…may involve additional mechanisms related to how autophagosomes might form…"

"…and targets it for degradation in autophagosomes."

"…monitor autophagy by measurement [of] the autophagosome marker LC3-II…"

"…involves the formation of a closed, double-membrane structure, called the autophagosome."

"…autophagosomes can be activated by…"

"…and the number of autophagosomes…"

"…accumulation of LC3-II to the autophagosome membrane…"

"…autophagosome formation in mammalian cells…"

"…for autophagosome biogenesis during starvation."

"…accumulation of autophagosomes and autolysosomes."

"…autophagosome-associated LC3-II…"

"…buildup of autophagosomes…"

"…mechanism of autophagosome formation…"

In many of these cases, it would be possible to substitute "phagophore" for "autophagosome." For example, LC3-II localizes to the phagophore, we are interested in the mechanism of phagophore formation, etc. Next, I simply did a PubMed search for the title word "autophagosome" and found 116 hits compared to nine for "phagophore." In case the title requirement was too restrictive, I then did the same comparison searching for these terms in any field and came up with 728 for "autophagosome" versus 47 for "phagophore." These two ratios are quite similar, indicating an approximately 14-fold bias for "autophagosome." (As an aside, when I carried out the latter search using "isolation membrane" I got 1,232 hits for this term in the title and 68,955 for any field; few of these have anything to do with autophagy, which is why I prefer the term "phagophore," as "isolation membrane" is too general—all organelles are surrounded by an isolation membrane). Now, considering all that I have said above, does this seem fair? I think not. Clearly, the phagophore is getting shortchanged.

I contend that, in the case of macroautophagy, it is the phagophore, not the autophagosome, that deserves our words of praise and continued attention. So, let me leave you with this final thought:

The autophagosome is large and round,
a structure that is quite sturdy and sound;
but its origin is the phagophore.
Thus, do I need to say anything more?