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The Golgi as a potential membrane source for autophagy

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In macroautophagy (hereafter autophagy), a morphological hallmark is the formation of double-membrane vesicles called autophagosomes that sequester and deliver cytoplasmic components to the lysosome/vacuole for degradation. This process begins with an initial sequestering compartment, the phagophore, which expands into the mature autophagosome. A tremendous amount of work has been carried out to elucidate the mechanism of how the autophagosome is formed. However, an important missing piece in this puzzle is where the membrane comes from. Independent lines of evidence have shown that preexisting organelles may continuously supply lipids to support autophagosome formation. In our analysis, we identified several components of the late stage secretory pathway that may redirect Golgi-derived membrane to autophagosome formation in response to starvation conditions.

Key words: lysosome, membrane biogenesis, protein targeting, secretory pathway, stress, vacuole, yeast

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Involvement of the Endoplasmic Reticulum (ER) and Mitochondria in Autophagosome Formation

Among all the membrane-bound organelles, the ER is the first one to be proposed as a membrane source for autophagosome formation. This hypothesis was initially supported by the identification of mammalian ER proteins on the forming autophagosomes, although other studies showed conflicting observations indicating that the phagophore lacks ER-resident proteins. In epithelial cells, the phagophores are frequently observed between the cisterns of rough ER. Recently, electron tomography shows that the mammalian ER is interconnected with the phagophores, and a subdomain of the ER forms a cradle-like structure to encircle the forming autophagosome. In the yeast *Saccharomyces cerevisiae*, autophagy is defective in strains with mutations in the early stage of the secretory pathway that is essential for ER-to-Golgi transport, suggesting the ER may contribute to autophagosome formation as a membrane source. Subsequent studies, however, provided some arguments against this proposition, suggesting that it may be just an indirect effect if an autophagy-essential protein is transported through the secretory pathway. Thus, researchers will have to differentiate whether the membrane flow or the cargo of the secretory pathway is required for autophagy.

Besides the ER, the mitochondria have also been speculated to be a membrane source during autophagy. Recently, researchers found that in mammalian cells the mitochondrial membrane is interconnected with associated autophagosomes, and fluorescently-labeled lipids are transferred from the mitochondria to the forming autophagosomes. In yeast, Atg9, an integral membrane protein cycling between the phagophore assembly site (PAS) and peripheral membrane structures, is hypothesized to be a lipid carrier that delivers membrane to the PAS to support phagophore expansion. Atg9 partially localizes adjacent to mitochondria based on fluorescence and electron microscopy, and the ultrastructural analysis shows that the Atg9-positive structures are clusters of vesicles and tubules. These clusters may translocate to, and/or form, the PAS during starvation conditions. Therefore, these mitochondria-related structures may contribute to autophagosome formation.

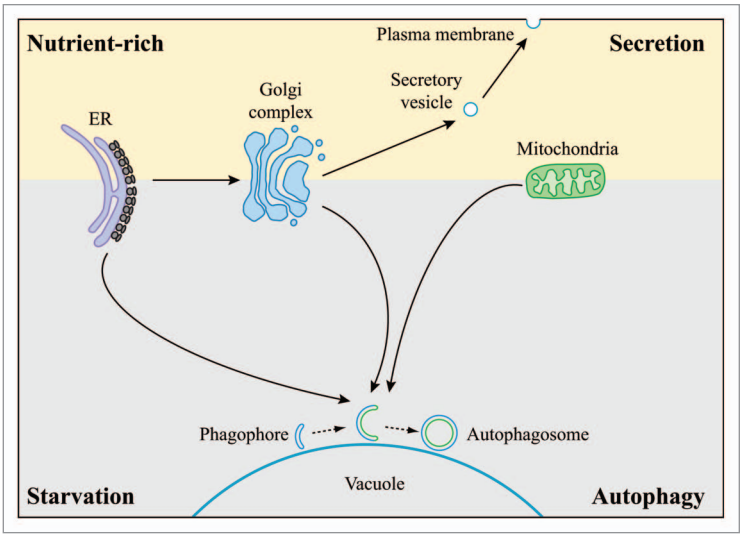

Figure 1. Schematic representation of membrane flow in the secretory pathway and autophagy. In nutrient-rich conditions, secretory vesicles are sorted from the Golgi to the plasma membrane for secretion. Upon starvation conditions, the Golgi-derived membrane flow may be redirected to the phagophore assembly site. Besides the Golgi, other organelles, such as the ER and possibly the mitochondria, are also involved in this process.

The Golgi as a Potential Membrane Source for Autophagosome Formation

The Golgi complex is an intricate network of cisternae, tubules and associated vesicles. Golgi-derived vesicles are sorted toward the plasma membrane and other intracellular organelles. Similar to the situation with the mitochondria and ER, there is substantial controversy concerning the role of the Golgi in autophagy. For example, in some cases mammalian Golgi markers are present on forming autophagosomes, but other studies fail to detect them.

In our analysis, we identified two post-Golgi Sec proteins essential for autophagy and proposed that membrane flow from the Golgi can be redirected to the PAS for autophagosome formation (**Fig. 1**). Compared to the early Sec proteins, Sec2 and Sec4 may have a more direct function during autophagosome formation. Based on our current knowledge, Sec4, a Rab GTPase, and its GEF, Sec2, are only involved in the polarized sorting of secretory vesicles from the Golgi to the plasma membrane (PM). We showed that protein secretion is downregulated to a very low level when autophagy is induced by starvation conditions, suggesting that Golgi-to-PM secretory flow is not required for autophagy. For this reason, the autophagy defect in *sec2* and *sec4* mutants is not due to a block in secretion but rather is a direct result from the dysfunction of Sec2 and Sec4.

In our present model, Atg9 movement to the PAS may represent the membrane supply to the autophagosome formation site. In both *sec2* and *sec4* mutants, Atg9 transport to the PAS is less efficient, suggesting the membrane supply is limited in these mutants. Sec2 and Sec4 are associated with secretory vesicles during exocytosis. They may be recruited to the Atg9-positive vesicles and direct these vesicles to the PAS, which is still far from proven. Furthermore, the exit of vesicles from the trans-Golgi is indispensible for autophagosome formation, which also supports our hypothesis that the Golgi-derived membrane flow participates in the vesicle formation step of autophagy.

Additional evidence also implies the possible role of the Golgi complex in autophagosome formation. Recently, it is reported that subunits of the conserved oligomeric Golgi (COG) complex are required for vesicle formation in both the Cvt pathway and autophagy. Additionally, Sec7, a late Golgi GEF protein essential for the secretory pathway, is also indispensible for autophagy. Further analysis shows that Sec7 participates in autophagy by acting on the Arf GTPase, again suggesting that post-Golgi transport is necessary for phagophore expansion. In combination with our data on post-Golgi Sec proteins, these results strongly indicate that Golgi-derived membrane flow is switched from exocytosis to the autophagy pathway during starvation conditions.

In conclusion, multiple membrane sources may be implicated in autophagosome formation. Blockage of membrane supply from any one of these sources leads to a failure in vesicle formation, suggesting the diversity of their functions in autophagy. It is not clear whether each membrane source participates in a particular stage of autophagosome formation, and how they coordinate to facilitate the robust induction of autophagy. In addition, there are still many unsolved questions regarding how the Golgi-derived membrane participates in autophagy. For instance, there is no direct evidence showing lipid transport from the Golgi to the PAS. In addition, it is not yet known how Sec4 recognizes the PAS. For example, is there an effector protein of Sec4 at this location? Further analysis is needed to answer these and similar questions.