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Autophagy

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Introducing secretory reticulophagy/ER-phagy (SERP), a VAMP7-dependent pathway involved in neurite growth

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Abstract

Together with the proteasome, macroautophagy is a main pathway for the degradation of intracellular elements. Endoplasmic reticulum (ER)-autophagy *i.e.* reticulophagy/ER-phagy leads to the encapsulation of pieces of the ER in forming autophagosomes. This is generally followed by fusion with lysosomes and degradation of these ER components by lysosomal hydrolases. Recent work by our group shows that ER elements could also be incorporated into late endosomes and later be released by a secretory mechanism which we will herein refer to as secretory reticulophagy/ER-phagy (SERP). In the absence of macroautophagy, such as by knocking out *Atg5*, SERP is more efficient, leading to an increased secretion of MAP1LC3B-II and LC3-interacting region (LIR)-containing proteins of the ER, reticulons and atlastins. In this scenario, neurites grow longer and neuronal polarity is altered. In the absence of SERP, such as by knocking out *Vamp7*, secretion of MAP1LC3B-II, ER-LIR containing proteins and neurite growth are severely inhibited. We argue that SERP might be a main secretory

mechanism bypassing the Golgi apparatus, and that it is particularly active and important in neurite growth.

Degradative reticulophagy

Eliminating excess of ER by reticulophagy was initially identified in yeast as part of the unfolded protein response (UPR). In the quintessential reticulophagy, ER LIR-containing proteins bind MAP1LC3-II in the phagophore membrane and this drives the incorporation of ER components within the autophagosome. Fusion with a lysosome then leads to degradation of the ER elements (Figure 1). Reticulophagy receptors are proteins of the ER such as reticulons and atlastins which have LIRs, small peptidic motifs, most often [W/F/Y]xx[L/I/V], which allow for the recruitment of Atg8-family members such as MAP1LC3s and GABARAPs. Another mechanism, ER-to-lysosome-associated degradation (ERLAD), leads to the direct engulfment of a piece of ER in an endolysosome. This pathway also involves ER LIR-containing proteins and requires ESCRT-III to close the late endosome membrane around the engulfed piece of ER.

Latest work: role of VAMP7 in SERP, a secretory mechanism involved in neurite growth

In our latest publication [1], we found that axonal growth is affected by partial starvation and drugs which activate (rapamycin, resveratrol, torin-1) or inhibit (spautin-1) autophagy. Partial starvation, achieved by diluting amino acids, vitamins and N2 supplement of the neuronal culture medium, stimulates axonal growth. Autophagy activators stimulate axonal growth, without affecting axonal polarization. Paradoxically, spautin-1 also stimulates axonal growth but in a radically different manner because it also inhibits axonal polarization with treated neurons showing multiple axons instead of only one. We then took advantage of PC12 cells, a model of neuronal-like cells which can be easily genetically manipulated. We characterized *Vamp7*- and *Atg5*-knockout (KO) cells. VAMP7, a secretory late endosome vesicular SNARE,

was indeed previously shown to mediate NGF-evoked neurite growth in PC12 cells. VAMP7 had previously been implicated in autophagy, particularly in the fly where VAMP8 is not present. ATG5 is an essential early component of macroautophagy. We confirmed that *Vamp7* KO PC12 cells show decreased, whereas *Atg5* KO cells show increased, neurite growth. Rapamycin only increases growth of the longest neurite and this effect is not seen in *Vamp7* KO cells.

Through lipidomics and proteomics of cell content and secretome, validation of hits by western blotting, and exploration of the effect of cellular expression of an anti-VAMP7 synthetic antibody and the Longin amino-terminal domain of VAMP7 lead us to the concept that ER elements destined to be degraded by reticulophagy are also released in large extracellular vesicles. This secretory route, which we refer to as secretory reticulophagy/ER-phagy (SERP), allows for the release of reticulophagy-related molecules RTN1 (reticulon 1), RTN3 and RTN4, ATL1 (atlastins GTPase 1) and ATL3, CALCOCO1, and MAP1LC3B-II (Figure 1). SERP is greatly enhanced when degradative reticulophagy is blocked such as by knocking out *Atg5* or impairment of lysosomal pH by treatment with the V-ATPase inhibitor bafilomycin A_1. We also show that SERP is strongly inhibited by knocking out *Vamp7*. Expression of a VAMP7 synthetic antibody prevents starvation-induced axonal growth, and expression of the Longin domain strongly affects the subcellular localization of RTN3 upon rapamycin treatment.

Outstanding questions

First, two main mechanisms could lead to the presence of ER LIR-containing transmembrane proteins, inside $VAMP7^+$ late endosomes. The ER-derived vesicles which contain these molecules could either be engulfed as in ESCRT-III-dependent ERLAD or undergo SNARE-dependent fusion with the late endosome. In the latter, VAMP7, which is localized at the late endosome and is able to interact with ER target SNAREs STX5A (syntaxin 5A) and SNAP47,

could mediate the fusion reaction (Figure 1). However, this latter mechanism would not account for the presence of RTN3 in large extracellular vesicles and it would rather be expected in 'classical' exosomes if it reached the late endosomal limiting membrane. In any case, there is a need for direct evidence, and our lipidomic analysis of *Atg5* and *Vamp7* KO cell contents might provide some leads.

Second, how MAP1LC3B-II reaches secretory late endosomes remains to be explored. At present, several mechanisms have been proposed through binding of LIR-containing proteins, lipidic enzymatic modification on late endosomal membranes or transport from autophagosomes.

Third, does SERP only coincide with neurite growth or is it a basic mechanism mediating plasma membrane growth? Neurite growth is a massive constraint for neuronal cells because it means adding up to 200,000x times the surface of its plasma membrane, an enormous burden of anabolism. It would be logical that the upregulation of ER that is required to support this increased synthesis of proteins and lipids is accompanied by a regulated elimination of potential ER surplus. Degradative reticulophagy might not be sufficient, thus SERP might be important to complete the cell capacity to regulate ER expansion. It is equally sound to consider that SERP contributes to plasma membrane growth through addition of lipids and proteins of endosomal and ER origin. In addition, we found that *Atg5* KO PC12 cell conditioned medium inhibits the growth of naïve PC12 cells. This suggests that the SERP secretome might carry some autocrine and paracrine messengers acting to generate a retrograde feedback mechanism preventing the kind of overgrowth that would otherwise allow for multiple axons and loss of neuronal polarity, as seen when autophagy is impaired.

Fourth, we found secretion of a short form of RTN3, RTN3A1, whereas the long form, RTN3L, was previously associated with degdradative reticulophagy, and both have LIRs (Figure 1). Is there a signal which differs between the two isoforms?

Finally, SERP provides a molecular and cellular framework for unconventional secretion bypassing the Golgi apparatus. Our secretomics suggest that proteins without a LIR could also be secreted by SERP in large extracellular vesicles (Figure 1). SERP might thus appear as an important route for cell secretion and cell-cell communication. Exploration of SERP in animal models will be important to have a clearer view on the potential physiological impact in normal and pathological brain development and function, and neuropathologies.

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Conflict of Interests

All authors declare no conflict of interest.

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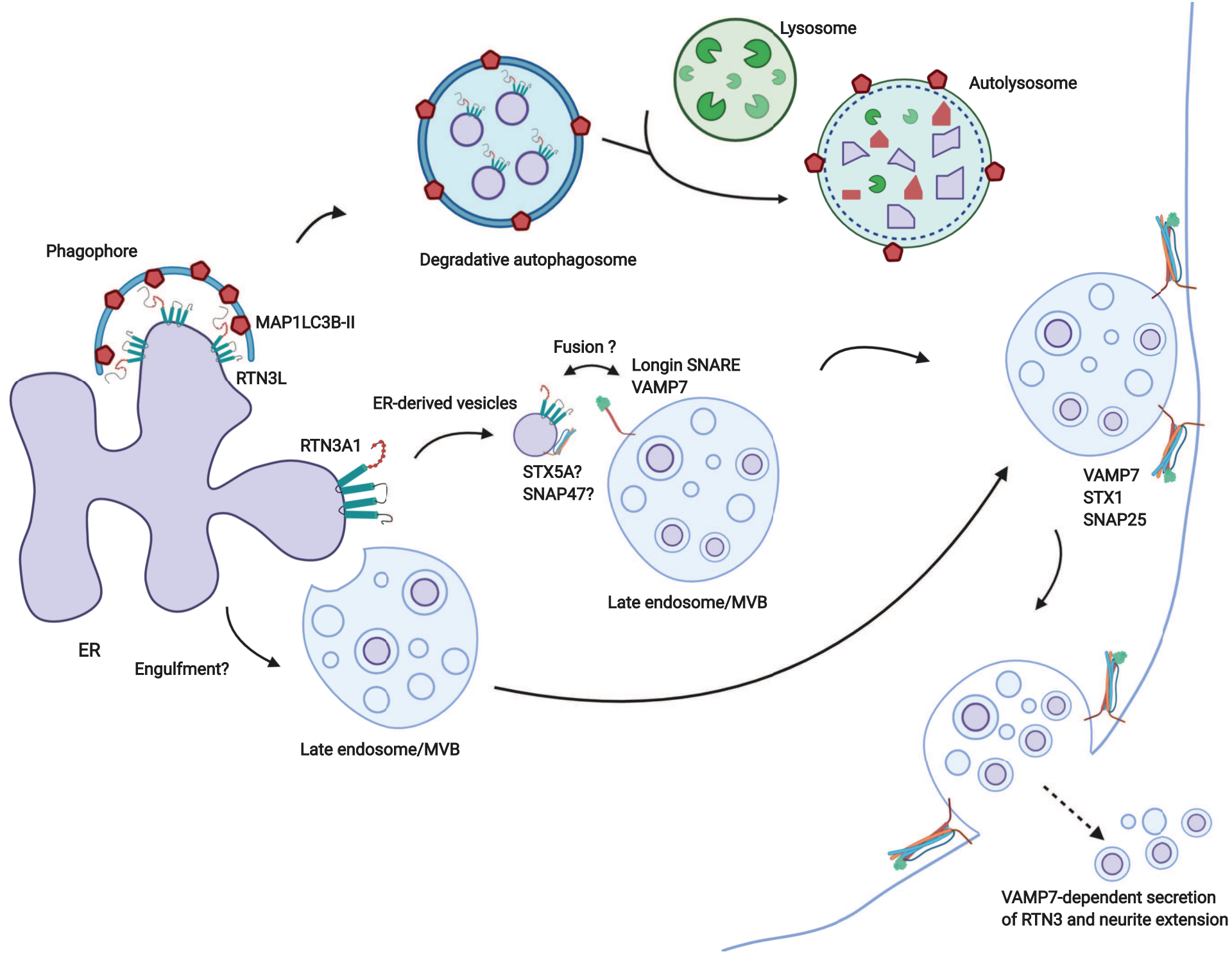
Figure 1. Working Model of VAMP7-dependent secretory reticulophagy/ER-phagy (SERP). ER adaptor proteins such as RTN3L have LC3-interacting regions, which can interact with MAP1LC3/GABARAP and help in packaging of ER fragments inside nascent autophagosomes. These degradative autophagosomes containing ER components then fuse with lysosomes wherein degradation of ER cargo and recycling takes place. In an alternative route, ER fragments containing RTN3A1 could become associated with late endosomes/multivesicular bodies (MVBs) via either direct engulfment or the SNARE-dependent fusion of ER-derived vesicles with late endosomes. The Longin SNARE VAMP7 is present on late endosomes/multivesicular bodies (MVBs) and might associate with ER-SNAREs SNAP47 and STX5A to facilitate this fusion. These MVBs fuse with the plasma membrane in a VAMP7-dependent manner thereby secreting ER cargo such as reticulons in the extracellular space. This secretion aides in the expansion of the plasma membrane and neurite growth. Figure created with biorender.


Lysosome
Autolysosome
Degradative autophagosome
Phagophore
MAP1LC3B-II
RTN3L
RTN3A1
ER
Engulfment?
ER-derived vesicles
Fusion ?
Longin SNARE
VAMP7
STX5A?
SNAP47?
Late endosome/MVB
Late endosome/MVB
VAMP7
STX1
SNAP25
VAMP7-dependent secretion
of RTN3 and neurite extension

Figure