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Getting intimate: Lysosomes and ER rendezvous to control autophagy

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ABSTRACT

The endoplasmic reticulum (ER) is the source of lysosomal calcium. The finding that the protein TMBIM6 –a putative ER calcium channel and cell death regulator –promotes calcium transfer from the ER to lysosomes to induce autophagy uncovers a missing piece in the puzzle of inter-organelle communication.

The endoplasmic reticulum (ER) is the main intracellular calcium reservoir. Calcium is released from the ER in part through inositol trisphosphate receptors (IP_3R), which are also present in ER specialized subdomains known as mitochondria-associated ER membranes or MAMs. The ER forms an extensive tubular network and physically interacts with the vast majority of cellular organelles through membrane contact sites (MCS). Early and late endosomes are strongly tethered to the ER, functioning as focal points for lipid exchange, cargo sorting, endosome trafficking, and fission [1]. Interactions between the ER membrane and lysosomes have recently proved to be crucial for lysosomal calcium homeostasis and signaling.

Early studies [2,3] showed that the disruption of the lysosomal pH gradient led to an increase in the concentration of cytosolic calcium due to a release from the ER, suggesting that functional lysosomes are able to sequester calcium. Indeed, inhibition of all three IP_3R isoforms completely abrogates lysosomal calcium refilling, indicating that the ER is the main source of lysosomal calcium [4]. IP_3Rs are preferentially localized to ER-lysosome MCS [5], where they mediate the selective transfer of calcium to lysosomes. However, when calcium is released by a more general mechanism, such as by inhibiting the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), lysosomal pH dissipation still increases cytosolic calcium, even in cells that are deficient of all IP_3Rs' isoforms. This suggests that there are additional, unknown ER calcium leak channels that are able to deliver calcium to lysosomes waiting to be identified. Very recently, Kim and colleagues [6] identified a new component of this pathway.

The authors discovered that the ER protein Transmembrane BAX Inhibitor Motif Containing 6 (TMBIM6, aka BI-1) –a factor previously implicated in the regulation of ER calcium content and cell death –promotes ER to lysosome calcium transfer, enhancing autophagy and lysosome biogenesis (Figure 1). The authors showed that inhibition of TMBIM6 or IP_3R resulted in decreased lysosomal calcium concentration, with the double inhibition having an even larger effect. Increased lysosomal calcium by TMBIM6 overexpression was still observed in IP_3R triple knockout (TKO) cells, suggesting parallel and independent functions for these proteins in the regulation of lysosomal calcium stores.

Tethering and formation of ER-lysosome MCS are accomplished by the establishment of different protein complexes, including the ER embedded proteins vesicle associated membrane protein-associated protein A (VAP-A) and Protrudin, which associate with the lysosomal protein Rab7. Using a previously reported TMBIM6 channel mutant, the authors showed that calcium released through TMBIM6 promotes the tethering and association of lysosomes to the ER membrane. How does calcium regulate organelle tethering? The exact mechanism is not completely understood, but calcium released from endosomes through the two-pore channel 1 (TPC1) is essential for contact formation with the ER and a similar function for TPC2 has been suggested for ER-lysosome MCS [7]. Thus, one possibility is that a higher number of ER-lysosome contacts are an indirect consequence of increased lysosomal

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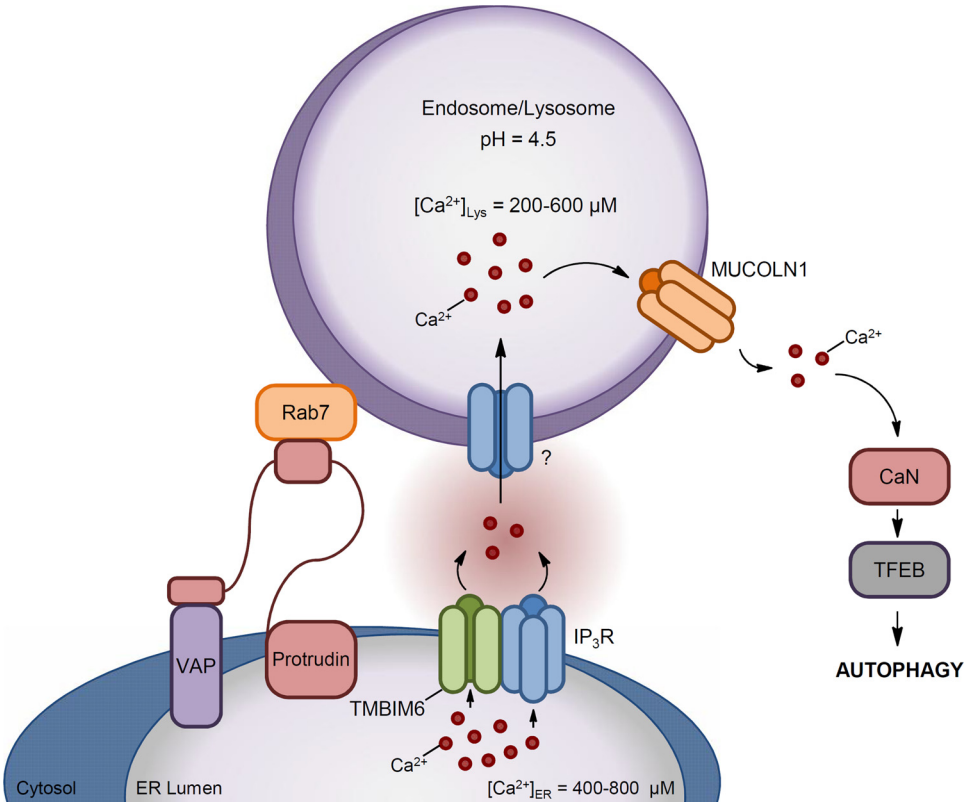

Fig. 1. ER-lysosome calcium transfer regulates autophagy. Kim et al. [6] report that the protein TMBIM6 promotes calcium transfer from the ER to the lysosome, regulating autophagy. This process occurs in two steps: first, calcium efflux through TMBIM6 leads to increased tethering of lysosomes to the ER membrane, resulting in a tight but persistent contact between these two organelles. Tethering is accomplished by the interaction of the ER-localized proteins vesicle-associated membrane protein-associated protein A (VAP-A) and Protrudin with Rab7, a lysosomal membrane-associated protein. Once in close proximity, calcium released from the ER by TMBIM6 and IP_3Rs leads to increased local cytosolic calcium concentration in the gap between these two organelles, which are sufficient to allow its uptake into the lysosome by a yet unidentified low-affinity calcium channel. Upon starvation, calcium released from the lysosome through MUCOLN1 channels activates the phosphatase Calcineurin (CaN). Calcineurin dephosphorylates and activates the transcription factor TFEB, a lysosomal master transcription factor that coordinates the expression of genes involved in autophagy and lysosomal hydrolases. TMBIM6 interacts with IP_3R to regulate calcium homeostasis at the ER (see the text).

calcium concentration and not vice versa. The authors did not perform organelle dynamic studies to assess the relationship between the persistence of ER-lysosome contacts and lysosomal calcium concentration.

What are the consequences of increased lysosomal calcium? A previous report [8] showed that, upon starvation, calcium is released from the lysosome through the mucolipin 1 (MCOLN1, aka TRPML1) cation channel, resulting in the activation of Calcineurin (CaN), a calcium and calmodulin-dependent serine/threonine phosphatase. CaN dephosphorylates the transcription factor IIB (TFEB), promoting its nuclear translocation and the subsequent upregulation of genes involved in autophagy and lysosomal biogenesis. In addition to confirm the critical role of MCOLN1 in autophagy induction, Kim and colleagues showed that the downregulation of TMBIM6 completely abolished lysosomal calcium release, CaN activation and TFEB nuclear translocation, resulting in impaired induction of autophagy. Given the essential role of TMBIM6 in the induction of autophagy, the authors examined its impact in the degradation of autophagy substrates. Inhibition of either TMBIM6 or IP_3R at the ER and MCOLN1 at the lysosome led to increased content of huntingtin aggregates, suggesting that the ER-to-lysosome calcium pathway support basal levels of autophagy to degrade harmful protein aggregates. The same group also reported that TMBIM6 overexpression alters lysosomal pH and lysosomal morphology, possibly involving the vacuolar H^+-ATPase [9]. The oligomerization of TMBIM6 was also shown to be dependent on pH. Since lysosomal calcium is coupled to pH control, it remains to be determined if the effects of TBMIM6 on pH are secondary to the regulation of lysosomal calcium.

Since TMBIM6 interacts with and sensitizes IP_3R [10], an interesting possibility is that TMBIM6 associates with clusters of IP_3Rs at ER-lysosome MCS, generating microdomains of high calcium concentration at the interface of these two organelles, allowing its uptake by a yet unidentified low-affinity lysosomal calcium channel. This process resembles ER-to-mitochondria calcium transfer at the MAMs, where the formation of calcium microdomains creates the conditions for the low-affinity mitochondrial calcium uniporter (MCU) to incorporate calcium into the matrix.

The identification of TMBIM6 as a critical regulator of ER-lysosomal MCS and lysosomal calcium homeostasis opens up interesting possibilities for future investigations into the role of TMBIM proteins in the regulation of inter-organelle communication. TMBIM6 is a member of the evolutionary conserved TMBIM superfamily, composed by at least five additional members [10]. Functionally, these proteins are characterized as pH-dependent calcium channels and localize to the membranes of different intracellular organelles, including lysosomes, the Golgi apparatus and the mitochondria, where they participate in different stress response pathways. The role that these proteins play in MCS formation and calcium transfer between different organelles remains completely unexplored.

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