RESEARCH HIGHLIGHT

TMED9–SEC12, an important "contact" for autophagy

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Autophagosome biogenesis occurs via a complex mechanism that involves membranes from different subcellular compartments. Here, we review an elegant study from Li et al. describing a new type of ERGIC–ERES membrane contact mediated by TMED9 and SEC12 that leads to the formation of COPII-coated vesicles essential for the formation of the autophagosome, and attempt to reconcile their findings with our studies proposing that the recycling endosome is a core platform for autophagosome biogenesis.

Macroautophagy (hereafter autophagy) is a cellular process where organelles and cytoplasmic proteins are engulfed into a double-membrane structure (the autophagosome) and delivered to lysosomes for degradation. Autophagy is crucial for the maintenance of cellular homeostasis and buffers against various stresses and nutrient deprivation. Autophagosome formation is a complicated process that involves different membranes from different subcellular compartments.[1]

The process of autophagy was originally described in yeast and involves the orchestrated action of multiple protein complexes. More than 40 core autophagy proteins have been identified in yeast and mammals, including so-called autophagy-related proteins (ATGs). These proteins regulate the different stages of autophagosome formation. Crucial steps in the process include PI3P generation by a kinase complex including VPS34 and Beclin 1, which recruits the PI3P-binding protein, WIPI2, which, in turn, recruits the ATG5–ATG12–ATG16L1 complex that is crucial for conjugation of LC3 family members to nascent autophagosome membranes, a defining step in autophagosome biogenesis.

In addition, it is clear that membrane trafficking between different subcellular compartments is essential for autophagosome biogenesis,[2] including endoplasmic reticulum (ER)-related compartments. Newly synthesized proteins destined for conventional secretion are packed in COPII-coated vesicles in an ER subcompartment, ERES (ER exit sites), and moved to the ER–Golgi intermediate compartment (ERGIC) before they are transferred to the Golgi complex.[3] Previously, Ge et al.[4] performed an in vitro reconstitution assay and proposed that the ERGIC is also necessary for LC3 family conjugation to phosphatidylethanolamine in nascent autophagosome membranes. Starvation, a primordial autophagy stimulus, activates phosphatidylinositol 3-kinase (PI3K) that induces the recruitment of COPII to the ERGIC complex, which, in turn, generates small vesicles that have the capacity to enable membrane conjugation of LC3 and/or serve as membranes that have a high capacity to be conjugated with LC3.[4,5]

In a recent paper published in *Cell Research*, Dr Ge's group performed mass spectrometry analysis of ERGIC-resident transmembrane proteins and identified TMED9 as the only transmembrane emp24 domain-containing (TMED) protein with an autophagic phenotype: overexpression of TMED9 induces autophagy and depletion of the protein compromises LC3 membrane conjugation.[6] TMED9 depletion not only affects canonical autophagy, but also selective autophagy processes, like mitophagy (degradation of mitochondria) and aggrephagy (degradation of aggregate-prone proteins). Using different morphological approaches (STORM, SD-SIM and EM-tomography), the authors showed that TMED9 regulates the contacts between ERGIC and ERES and its depletion affects the trafficking between the two compartments.

The counterpart of TMED9 on the ERES is SEC12 and the two proteins interact via their cytoplasmic tails. Starvation does not affect TMED9 localization but increases the interaction of the two proteins. Previously, Ge et al.[5] found that relocation of COPII machinery to the ERGIC compartment leads to generation of "ERGIC-derived" COPII vesicles necessary for LC3 lipidation and that starvation induces the enlargement of this compartment where ERES colocalizes with SEC12.

In the present paper, the authors conclude that ERGIC-TMED9 and ERES-SEC12 interact to enable contacts between these two compartments. In vitro experiments using giant unilamellar vesicles (GUVs) revealed that the interaction between the two proteins is sufficient to drive the contact between ERGIC and ERES. Overexpression of the TMED9 C-terminal portion abrogates the interaction between GUVs and the two compartments. Interestingly, as overexpression of the same mutant decreases autophagosome formation, this argues that the interaction between ERGIC-TMED9 and ERES-SEC12 is important for autophagosome biogenesis.

CTAGE5 is a SEC12-binding protein that is required for ERES enlargement under starvation conditions, SEC12 delocalization to the ERGIC and autophagosome biogenesis. The autophagic protein FIP200 binds directly (and independently of the ULK1 complex) to SEC12, and thereby facilitates the starvation-induced enlargment of the ERES. FIP200 or CTAGE5 depletion disrupts ERES enlargement and abolishes ERES–ERGIC contacts.

This interesting and elegant paper adds important components to the understanding of autophagosome by identifying and characterizing ERGIC–ERES contacts as critical components of the process.

Our data suggest that the membrane platform where LC3 is conjugated to is the RAB11A recycling endosome compartment that is adjacent to ER.[7,8] Indeed, RAB11A, a marker protein for recycling endosomes, is a coincident detector for WIPI2 (along

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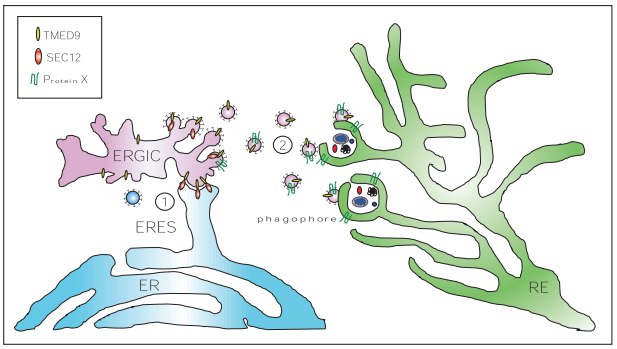

Fig. 1 ERGIC–ERES contact formation. (1) The TMED9–SEC12 interaction led to the formation of the ERES–ERGIC contact site. (2) COPII vesicles bud from this compartment and are delivered to the sites of autophagosome formation. Protein X represents a putative regulator of autophagosome formation. RE, recycling endosome.

with PI3P), and is essential for membrane recruitment of this protein, LC3 membrane conjugation and autophagosome biogenesis in cells.[7] We can reconcile our data with those of Ge and colleagues[4,5,9]—their in vitro reconstitution data could be interpreted as showing that important components of the LC3-conjugating machinery are associated with ER-related compartments, but do not require that the compartment conjugated with LC3 family members in living cells is necessarily ER-related (Their experiments[4,9] did not uncouple the capacity of a membrane fraction to induce LC3 conjugation versus the likelihood that a particular membrane fraction would be receptive to conjugation). Indeed, we found that ATG3, a key enzyme required for LC3 conjugation, is much more strongly associated with ER-associated compartments than the recycling endosome.[7] On the other hand, we found much stronger recycling endosome association using both microscopy and biochemistry with PI3K complex members (Beclin 1 and VPS34), the PI3P-binding protein WIPI2, which determines the sites of LC3 conjugation and LC3 family members themselves. Thus, we speculate that the vesicles generated from ERGIC–ERES contacts either provide critical enzymes like ATG3 to enable LC3 conjugation and/or may fuse to sites on the recycling endosomes to render them more competent to be conjugated (Fig. 1).

In future, it will be important to identify which key protein/s required for autophagy are transported by the COPII vesicles enabled by the ERGIC–ERES contacts. Moreover, it will be interesting to understand whether such factors are transported directly (or indirectly through other compartments) from the ERGIC–ERES contact sites to the platform where the autophagosomes are formed. If the transport of such factors is direct, this may help cells respond rapidly to stresses like starvation that stimulate autophagy.

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COMPETING INTERESTS

D.C.R. is a consultant for Drishti Discoveries, Abbvie, PAQ Therapeutics, Aladdin Healthcare Technologies SE and Nido Biosciences.

ADDITIONAL INFORMATION

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