A novel regulator of autophagosome biogenesis and lipid droplet dynamics

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Autophagosome biogenesis is the key event associated with the stress-responsive autophagic pathway, allowing the capture of specific cargoes and their delivery to the lysosomal degradative compartment. Although the endoplasmic reticulum (ER) appears to be central for the assembly of autophagosomal membranes, it is also involved in several events regulating trafficking and local signaling, e.g., the establishment of contact sites with other organelles, the vesicular transport to the Golgi apparatus, and the biogenesis and turnover of lipid droplets. In this issue of *EMBO reports*, Moretti *et al* [1] identify the ER transmembrane protein TMEM41B as a novel regulator of autophagosome biogenesis and unravel its involvement in lipid droplet dynamics, also highlighting the role of ER components at the interface of lipid metabolism and regulation of autophagy.

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Autophagy is a lysosomal-associated intracellular degradative pathway which participates in cellular homeostasis, especially in stress situations such as nutritional deprivation, microbial infection, or mechanical constraints [2]. The autophagic program mobilizes several membrane compartments and a plethora of proteins, including the autophagy-related proteins (ATGs), to initiate and complete the formation of autophagic vesicles known as autophagosomes. The latter are responsible for the selective and non-selective sequestration of intracellular material (such as protein aggregates and organelles) prior to their delivery to lysosomes in which final

degradation and recycling of molecular components take place. Despite critical recent advances in the field, autophagosome biogenesis is still a poorly understood phenomenon, in particular at the level of signaling and membrane dynamics [3]. Upon stress sensing, most autophagic responses will require the ULK1 complex, which communicates permanently with the nutrient sensor mTOR. ULK1 in turn promotes the mobilization of several ATG proteins, including the class III PI3Kinase complex (composed of VPS34, VPS15, beclin 1, and ATG14). Activation of the class III PI3Kinase leads to local phosphatidylinositol-3-phosphate (PI3P) synthesis on ER subdomains. This allows the recruitment of PI3P-binding proteins such as DFCP1 and WIPI, which will trigger autophagosome biogenesis. Local PI3P synthesis is thus considered as a pivotal molecular event to complete the autophagic process, notably by allowing LC3 lipidation and targeting to autophagosomal membranes. This occurs via the ATG5-12-16L1 complex, which is itself recruited to PI3P-positive domains via the PI3P-binding protein WIPI2. Once this pathway is activated, a pre-autophagosomal structure called phagophore arises in the surrounding cytoplasm from membrane cradles [4]. After the capture of autophagic cargoes by receptors such as P62 or NDP52, the phagophore will mature into an autophagosome by self-closure, a process likely mediated by membrane fission [5]. While the ER membrane appears to be essential for phagophore biogenesis, other membrane sources participate probably in the process directly or indirectly, via membrane contact sites initiated by the ER. Thus, identification of ER transmembrane proteins implicated in these early steps of

autophagy regulation is essential to decipher the molecular events involved in the mobilization and rearrangement of membranes occurring during phagophore formation.

Moretti *et al* [1] have now unveiled a so far unreported role for the ER transmembrane protein TMEM41B in autophagosome biogenesis. Using a FACS/CRISPR screen based on monitoring the turnover of the two autophagy-associated receptors NDP52 and p62 upon autophagy induction, the authors show that p62 and NDP52 accumulate in the absence of TMEM41B. TMEM41B knockout leads also to alterations in the autophagic flux, which is further confirmed by p62 exclusion from lysosomal organelles [1]. To decipher what step of autophagy is directly affected by the absence of TMEM41B, the authors analyze the behavior of proteins involved in (or reported to be associated with) the initiation of phagophore biogenesis. They show, using several different techniques, that the lipidated form of LC3 (i.e., LC3-II; the form of LC3 anchored to autophagosomal membranes) accumulates in cells lacking TMEM41B, as well as DFCP1 and WIPI2, two PI3P-associated proteins considered as markers of pre-autophagosomal structures. In parallel, Moretti *et al* describe a deficit in matured autophagosomes and the accumulation of electron-dense structures at the vicinity of ER membranes in TMEM41B KO cells, suggesting that the autophagic pathway is severely altered. However, the first events of pre-autophagy still occur, as illustrated by LC3-II and WIPI2 accumulation, which might indicate the persistence of stalled pre-autophagosomal membranes [1]. The authors thus suggest that TMEM41B controls membrane dynamics immediately after initiation of phagophore membrane biogenesis,

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presumably participating in the maturation and sealing of the nascent autophagosome. Because lipid metabolism and in particular lipid droplets (LDs) have been shown to be part of the chain of events leading to autophagosome biogenesis, the authors also monitor lipid droplets in TMEM41B KO cells and show that they are increased in size and total number compared to controls. Analysis of the lipid composition further revealed that mitochondrial oxygen respiration is affected, which suggests that TMEM41B might participate in mitochondria-associated β -oxidation of fatty acids. This unveils a new function for TMEM41B, where neutral lipids mobilized from LDs

could directly fuel the above-mentioned membrane remodeling events associated with autophagosome maturation and closure, as previously suggested [6,7].

These newly proposed functions of the ER transmembrane protein TMEM41B are related to the specific mobilization of neutral lipids from LDs at the vicinity of the ER membrane. This local LD metabolism will in turn favor phagophore growing and sealing (Fig 1), thus revealing that an ER-resident transmembrane protein can control some of the molecular events occurring on the isolation membrane, highlighting the interaction between the ER and autophagosomal membranes during the very first steps of

autophagy initiation. Similar observations have been previously reported for the transmembrane protein VMP1, which was shown to participate in phagophore biogenesis by recruiting the class III PI3Kinase in subdomains of the ER membrane, presumably via ER-driven contact sites with other organelles [8–10]. It is thus tempting to imagine that TMEM41B is an ER-based scaffolding regulator, acting in the assembly of autophagosomal structures by tethering locally LDs and ER membranes in a dedicated subcellular zone of the cytoplasm, presumably delimited by ER sheets or tubules, to favor lipid exchange from one membrane to another. Such a phenomenon could help to unravel the implication of ER-associated subdomains and contact sites in autophagosome biogenesis regulation.

Importantly, the work of Moretti *et al* puts ER transmembrane proteins that orchestrate the coordination of membranes in space and time on the list of putative regulators of the complex molecular machinery that drives autophagosome biogenesis.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figure 1. TMEM41B at the interface of LD mobilization and autophagosome biogenesis.

Both, lipid droplet (LD) and autophagosome assembly, take place at ER membrane. The KO of the TMEM41B ER transmembrane protein leads to selective autophagy dysfunction, characterized by the reduction in autophagosomal structures (normally mediated by the autophagy-associated receptors P62 and NDP52) and the accumulation of the early-stage regulatory protein WIPI2 at ER subdomains engaged in autophagosome biogenesis. In parallel, the KO of TMEM41B leads to severe LD accumulation and β -oxidation alterations. This suggests that TMEM41B participates in early stages of autophagosome biogenesis at the ER membrane via direct mobilization of neutral lipids from LDs.