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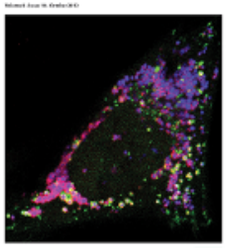

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Unconventional roles of nonlipidated LC3 in ERAD tuning and coronavirus infection

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Secretory and membrane proteins attain their native structure in the endoplasmic reticulum (ER). Folding-defective polypeptides are selected for degradation by processes collectively defined as ER-associated degradation (ERAD). Enhanced ERAD activity may interfere with protein biogenesis by inappropriately targeting *not-yet-native* protein folding intermediates for disposal. The regulation of ERAD is therefore crucial to maintain cellular proteostasis. At steady-state, select ERAD regulators are constitutively removed from the ER in a series of processes collectively defined as ERAD tuning. This sets the ERAD activity at levels that do not interfere with completion of ongoing folding programs. Our latest work highlights a crucial, autophagy-independent role of nonlipidated LC3 (LC3-I) as part of a membrane-bound receptor that insures the vesicle-mediated clearance of at least two ERAD regulators from the ER, EDEM1 and OS9. This pathway is hijacked by coronaviruses (CoV), and silencing of LC3 substantially inhibits viral replication.

ERAD Tuning and Cellular Proteostasis

A sophisticated quality control system operates in the ER to ensure fidelity of gene expression. Correctly folded and assembled proteins leave the compartment to be transported at their intra- or extracellular site of activity. Misfolded polypeptides are dislocated into the cytosol and degraded by the ubiquitin-proteasome system in a series of events collectively defined as ERAD.

Protein folding is assisted by molecular chaperones and folding enzymes. These are long-lived proteins ($t_{1/2}$ > 12 h) present at dauntingly high concentrations in the ER lumen. Selection for disposal of non-native polypeptides is regulated by ERAD factors, some of which are characterized by rapid turnover ($t_{1/2}$ < 2 h) that possibly determines their low intracellular concentration at steady-state. The selective removal at steady-state of ERAD factors from the ER, ERAD tuning, is instrumental to maintain cell and organism proteostasis by preventing the inappropriate degradation of not-yet-native folding intermediates that may occur when ERAD activity is aberrantly high.

LC3-I at the Limiting Membranes of ERAD Tuning Vesicles and of CoV-Induced DMVs

The low intralumenal concentration of the ERAD regulators EDEM1 and OS9 at steady-state is determined by their constitutive removal from the ER by a vesicular pathway, which is distinct from the conventional secretory pathway used for cargo protein export. Secretory vesicles have an average diameter of 60–70 nm and display coatomer proteins at their surface. The ER-derived ERAD tuning vesicles (EDEMosomes) containing EDEM1 and OS9 lack a recognizable coat and have a diameter of about 150 nm as estimated in immuno-electron microscopy. They may swell to up to 1 μm upon cell exposure to lysosomotropic agents, which prevents their fusion with acidic degradative organelles. The characterization of ERAD tuning vesicles in immunofluorescence and

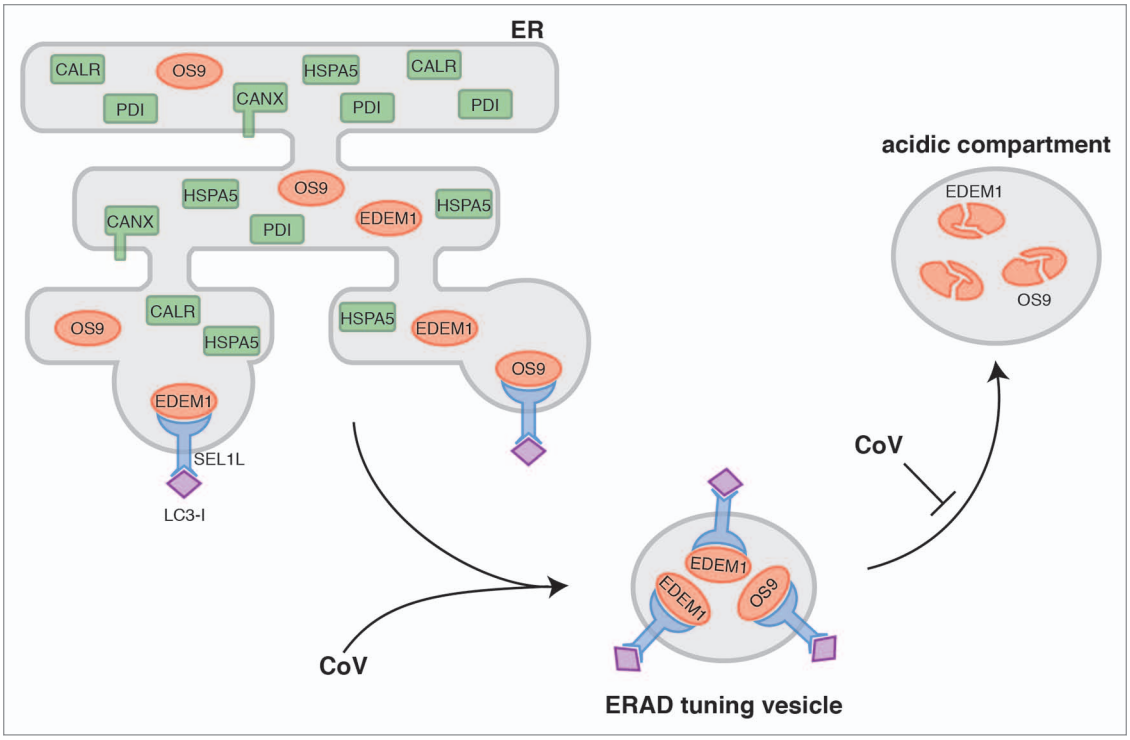

Figure 1. Model for the vesicle-mediated segregation of EDEM1 and OS9 from the mammalian ER. CANX: calnexin; CALR: calreticulin; HSPA5: 78 kDa glucose-regulated protein/BiP; PDI: protein disulfide isomerases. Note that it remains to be established whether LC3-I binds SEL1L directly (i.e., to the cytosolic, proline rich tail) or indirectly through an adaptor associated with the transmembrane or the cytosolic domains of SEL1L.

their separation from lighter secretory organelles and autophagosomes in isopycnic density gradients reveal the absence of conventional ER and secretory pathway markers, of cargo secretory proteins and of the autophagosome marker LC3-II. Rather, ERAD tuning vesicles contain the type I ER protein SEL1L and the luminal ERAD factors EDEM1 and OS9. LC3-I is non-covalently associated with their limiting membrane, which can be decorated with the $LC3^{G120R}$ mutant that cannot be lipidated, but not with the autophagosome marker GFP-LC3.

The presence of LC3-I is a characteristic that ERAD tuning vesicles share with ER-derived double-membrane vesicles (DMV) found in cells infected with the mouse hepatitis virus (MHV), a prototype coronavirus. The ER-derived DMVs in infected cells do contain a number of viral nonstructural proteins, replication complexes and double stranded RNA. Like ERAD tuning vesicles, the DMVs lack conventional ER and secretory pathway markers but do contain the host cell proteins EDEM1, OS9 and SEL1L. These findings led us to propose that CoV co-opt ERAD tuning vesicle-like structures as platforms for genome replication.

LC3-I as Part of an ERAD Tuning Receptor that Regulates ERAD Factor Clearance from the ER

In cells expressing misfolded polypeptides, the type I membrane protein SEL1L is part of a multimeric dislocation machinery built around E3 ubiquitin ligases, which dislocates misfolded proteins across the ER membrane for proteasomal degradation. However, at steady-state, at least part of the cellular SEL1L is disengaged from the dislocation machinery. The SEL1L ectodomain then associates with the lumenal ERAD factors EDEM1 and OS9 and delivers them into the ER-derived ERAD tuning vesicles. This is the initial step of a series of poorly characterized events that eventually lead to the disposal of EDEM1 and OS9 in acidic organelles that regulates the constitutive and rapid turnover of these crucial ERAD regulators. Significantly, LC3-I is part of the SEL1L–EDEM1 and SEL1L–OS9 complexes that segregate from the ER to enter the ERAD tuning vesicles (**Fig. 1**). The transmembrane and cytosolic portions of SEL1L are required and sufficient to confer LC3-I association and to drive segregation of proteins containing them (e.g., a calnexin ectodomain appended to the SEL1L transmembrane and cytosolic domains) in the ER-derived vesicles. It remains to be established whether LC3-I binds SEL1L directly (i.e., to the cytosolic, proline rich tail) or indirectly through an adaptor associated with the transmembrane or the cytosolic domains of SEL1L.

SEL1L or LC3 silencing inhibit the vesicle-mediated clearance of EDEM1 and OS9 from the ER. This enhances the overall ERAD activity at levels that eventually interfere with ongoing folding programs. SEL1L or LC3 silencing also inhibit MHV replication, thus confirming the hijacking of the ERAD tuning machinery by this CoV.

Misfolded Polypeptides Regulate ERAD Factor Turnover by Inhibiting the SEL1L–LC3-I-Mediated Clearance of ERAD factors from the ER

SEL1L–LC3-I-regulated clearance of EDEM1 and OS9 from the ER is reduced upon lumenal expression of misfolded polypeptides. Misfolded proteins engage EDEM1 and OS9 that must deliver them to the dislocation sites at the ER membrane and, at the same time, stabilize SEL1L in functional dislocation complexes. Under these circumstances, the formation of ERAD tuning complexes is disfavored and ERAD tuning is substantially reduced. Thus, misfolded polypeptides may regulate enhancement of ERAD activity by inhibiting the constitutive ERAD factor turnover occurring at steady-state. This is rapid and readily reversible because it does not rely on the activation of transcriptional/translational programs regulated by the unfolded protein response. The unfolded protein response may eventually intervene upon stronger and chronic ER stress signals.

Concluding Remarks

Future studies will shed light on the novel, autophagy-independent functions of LC3-I as a regulator of ERAD factor turnover and as a component of machineries hijacked by mammalian cell pathogens. It remains in fact unclear whether LC3-I serves as a molecular scaffold to induce the budding of the ERAD tuning vesicles and of the similar vesicles that contain the replication complexes in virus-infected cells and/or whether LC3-I functions as an adaptor molecule to promote the movement of the ERAD tuning vesicles to the (still elusive) degradative compartment along microtubules.

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