Autophagy 6:7, 994-996; October 1, 2010; © 2010 Landes Bioscience

Autophagy-independent LC3 function in vesicular traffic

Cornelis A.M. de Haan,[1] Maurizio Molinari[2,3] and Fulvio Reggiori[4]

[1]Virology Division; Department of Infectious Diseases and Immunology; Utrecht University; Utrecht, Netherlands; [2]Institute for Research in Biomedicine; Bellinzona, Switzerland; [3]Ecole Polytechnique Fédérale de Lausanne; School of Life Sciences; Lausanne, Switzerland; [4]Department of Cell Biology and Institute of Biomembranes; University Medical Centre Utrecht; Utrecht, Netherlands

©2010 Landes Bioscience. Do not distribute.

Key words: LC3-I, EDEMosomes, ERAD tuning, double-membrane vesicles, coronavirus, autophagy

Submitted: 08/06/10

Revised: 08/09/10

Accepted: 08/11/10

Previously published online: www.landesbioscience.com/journals/autophagy/article/13309

DOI: 10.4161/auto.6.7.13309

*Correspondence to: Fulvio Reggiori, Cornelis A.M. de Haan and Maurizio Molinari
Email: F.Reggiori@umcutrecht.nl, C.A.M.deHaan@uu.nl, maurizio.molinari@irb.unisi.ch

Punctum to: Reggiori F, Monastyrska I, Verheije MH, Calì' T, Ulasli M, Bianchi S, et al. Coronaviruses hijack LC3-I-positive EDEMosome membranes for replication. Cell Host Microbe 2010; 7:500–8; PMID: 20542253; DOI: 10.1016/j.chom.2010.05.013.

As protein folding is an imperfect process, the endoplasmic reticulum (ER) contains folding as well as ER-associated degradation (ERAD) machineries. In order to prevent premature interruption of folding, ERAD regulators and effectors such as EDEM1 and OS-9 are selectively cleared from the ER in so-called EDEMosomes to downregulate the degradative activity. The mechanism by which EDEM1 and OS-9 are subjected to rapid turnover, also known as ERAD tuning, shows similarities with, but is clearly distinct from, macroautophagy. Positive strand RNA coronaviruses (CoVs) such as the severe acute respiratory syndrome (SARS)-CoV and mouse hepatitis virus (MHV), induce in infected cells the formation of autophagosome-like, double-membrane vesicles (DMVs) to which their replication and transcription complexes are anchored. While it seems clear that CoVs hijack ER-derived host cell membranes for replication, the mechanism by which these DMVs are assembled has remained completely mysterious.

In a recent collaboration between our laboratories, we have shown that while MHV replication is autophagy-independent, the autophagy marker protein LC3/Atg8 is associated with the CoV-induced DMVs. Yet, whereas autophagosomes contain the lipidated form of LC3, i.e., LC3-II, the DMVs induced upon infection with MHV are exclusively decorated with non-lipidated LC3, i.e., LC3-I. These observations resembled those reported for the ERAD tuning process, whereby a select population of ER chaperones is discarded by the autophagy-independent formation of ER-derived vesicles, which are also decorated with LC3-I. Hence, we studied the relationship between ERAD tuning and MHV replication in more detail. MHV infection interferes with ERAD tuning and results in the accumulation of the ERAD tuning substrates EDEM1 and OS-9 in the CoV-induced DMVs. EDEM1 and OS-9 themselves are, however, not required for MHV replication, in contrast to LC3, depletion of which blocked replication. The data lead us to postulate that CoVs hijack, in an autophagy-independent way, the LC3-I-positive EDEMosomes for the generation of DMVs that provide the membranous support for the coronaviral replication and transcription complexes.

These findings also unveiled a new autophagy- and lipidation-independent function of LC3 in vesicular trafficking. Yet, the role of LC3-I in the formation of both EDEMosomes and CoV-induced DMVs remains completely unknown. One speculative idea is that LC3-I acts as a vesicle coat protein as was already hypothesized for LC3-II. In such a scenario and similar to other vesicular transport pathways, a still elusive EDEMosome cargo receptor would bind EDEM1 and OS-9, segregate these lumenal chaperones in specialized ER regions and recruit cytosolic LC3-I (**Fig. 1A**). This latter step will be the key event required for the coat-driven formation of a carrier vesicle that removes EDEM1 and OS-9 from the ER. Thus, one possible way for CoVs to exploit the ERAD tuning machinery for generating its replicative DMVs would be to hijack the EDEMosome cargo receptor, perhaps by using one or more of its transmembrane

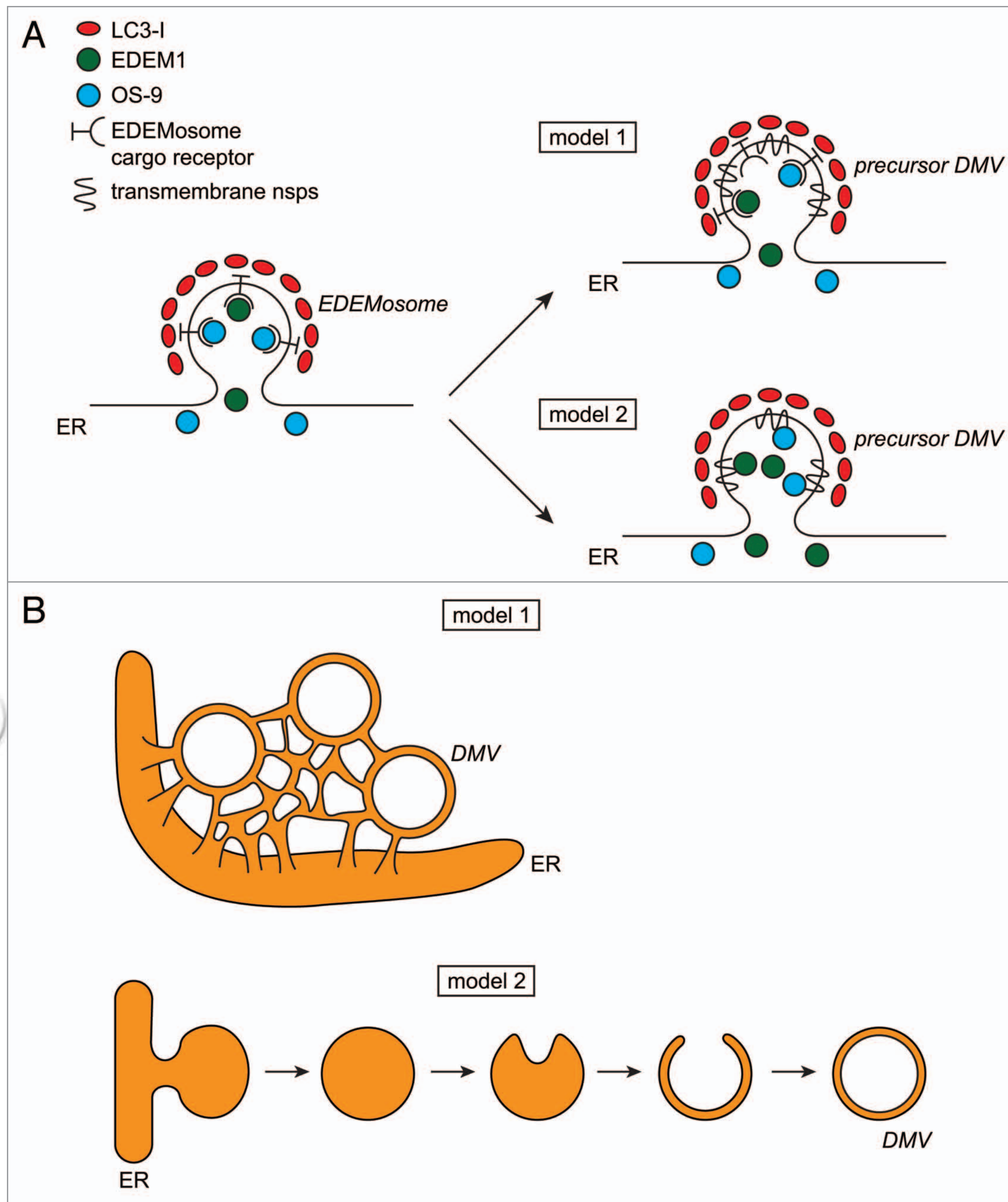

Figure 1. ERAD tuning and MHV-induced DMV biogenesis. (A) Early events in the biogenesis of EDEMosomes and of DMVs. EDEMosomes (left) and DMV precursors (right) are proposed to bud from the ER. Our models suggest a role of LC3-I as a vesicle coat protein. The cytosolic tail of an elusive EDEMosome cargo receptor (left and model 1) or viral nonstructural protein(s) (model 2) could recruit cytosolic LC3-I as a triggering event for formation of both EDEMosomes and/or DMV precursors. (B) DMV generation from the ER. Infection of cells leads to the formation of numerous interconnected DMVs (modified from the figure published in PLoS Biol by Knoops and coworkers) (model 1). Significantly, DMVs do not contain conventional ER markers but contain rapidly turned over chaperones such as EDEM1 and OS9 1. Based on the fact that DMVs often have an inward budding profile, DMVs could form by invagination and pinching off of the limiting lipid bilayer of a single-membrane vesicle through a process mediated by the viral proteins (model 2).

nonstructural proteins (nsps, e.g., nsp3, nsp4 and/or nsp6, **Fig. 1A**, model 1). Alternatively, these nsps act more directly by recruiting LC3-I and other vesicle coating factors (**Fig. 1A**, model 2). While the first model contemplates that EDEM1 and OS-9 end up in the DMVs through their association with the EDEMosome cargo receptor, the second does not explain the peculiar distribution of these two chaperones during MHV infection. A captivating possibility is that CoVs actively sequester EDEM1 and OS-9 into their DMVs in order to weaken the ERAD capacity of the host cell. At the peak of its replication, CoVs induce ER stress due to a sustained high production of viral components, including the three integral

membrane nsps and the three structural membrane proteins that are initially inserted in the ER lipid bilayer. One of the normal consequences of the induction of ER stress is the enhancement of ERAD. As this would hamper CoV replication by degrading part of the viral products, sequestering EDEM1 and OS-9, two positive regulators of the ERAD, could limit this deleterious cellular response.

Conventional budding of coated vesicles from donor organelles leads to the formation of single-membrane carriers. While it is still controversial whether EDEMosomes are single- or double-membrane vesicles, CoV-induced DMVs are undoubtedly composed of two concentric lipid bilayers. How are DMVs then generated from the ER? Based on electron tomography analyses of SARS-CoV-infected cells, it has recently been proposed that the reorganization of the membranes of this organelle, triggered by coronaviral proteins, leads to the formation of a reticulovesicular network (RVN) of modified ER that integrates convoluted membranes (CMs) and numerous interconnected DMVs (**Fig. 1B**, model 1). The mechanism underlying this model is, however, not clear. While performing an ultrastructural time-course analysis of MHV infection we could not observe particular budding profiles or connections between CMs and DMVs. Based on these data and on the observation that DMVs often have an inward budding profile in certain cell lines, we hypothesize that CoV-induced DMVs are formed by invagination and pinching off of the limiting lipid bilayer of a single-membrane vesicle through a process mediated by the viral proteins (**Fig. 1B**, model 2). In this scenario, the RVN, which becomes more prominent at the later stages of a CoV infection, might represent a side product caused by a massive production of viral components and/or by depletion of host cell factors required for the production of DMVs. Clearly, in this respect, it will be important to pinpoint the exact location of CoV RNA synthesis, being either the DMVs, CMs or both in cells infected with different CoVs.

Obviously the models presented here are still entirely hypothetical and the biogenesis of CoV-induced DMVs remains to be fully elucidated. In addition, LC3-I could have a different function in ERAD tuning and CoV replication than acting as a coat protein. Future studies on these two processes will shed light on the molecular role of LC3-I but also on the interrelationship between the ERAD tuning and CoV machineries.

Acknowledgements

The authors thank Peter Rottier for critical reading of the manuscript and Marc van Peski for assistance with the preparation of the figure. C.A.M.d.H. and F.R. are supported by the Utrecht University (High Potential grant). M.M. is supported by grants from the Foundation for Research on Neurodegenerative Diseases, the San Salvatore Foundation, the Swiss National Centre of Competence in Research on Neural Plasticity and Repair, the Swiss National Science Foundation and ONELIFE Advisors SA.

©2010 Landes Bioscience.
Do not distribute.