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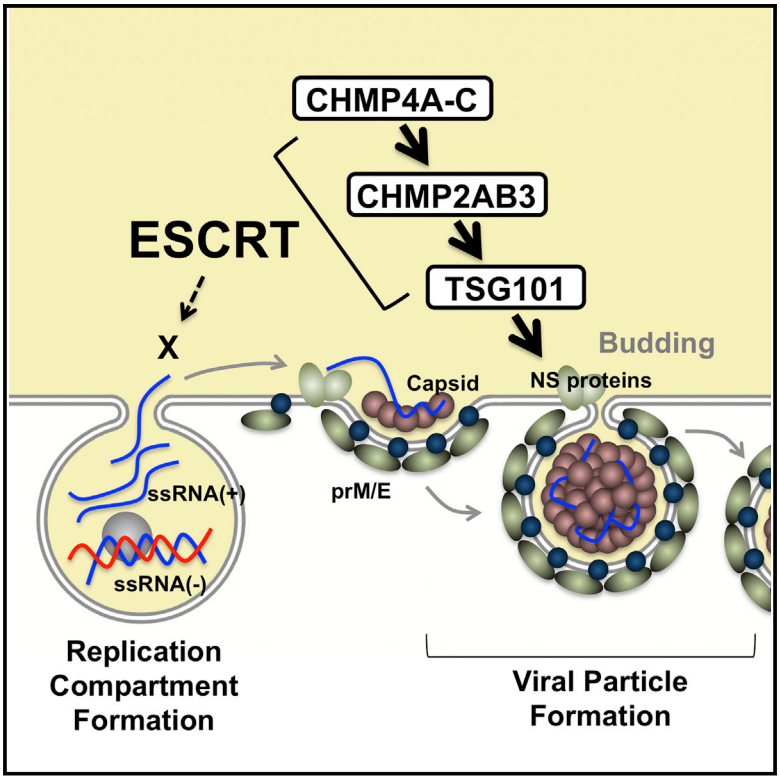
Unique Requirement for ESCRT Factors in Flavivirus Particle Formation on the Endoplasmic Reticulum

Graphical Abstract



Highlights

- Several ESCRT subunits are recruited to sites of flavivirus replication on the ER
- Systematic siRNA screening identifies a unique requirement for ESCRT factors
- Essential ESCRT subunits, VPS4 and ALIX, are dispensable for flavivirus replication
- ESCRT depletion affects virual budding but not the genome replication

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In Brief

Tabata et al. find that a unique subset of ESCRT family proteins is required for the formation of viral particles, but not for formation of the replication compartment. This unique ESCRT requirement implies the existence of ER-specific features for ESCRT-mediated membrane rearrangement.

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Unique Requirement for ESCRT Factors in Flavivirus Particle Formation on the Endoplasmic Reticulum

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SUMMARY

Flavivirus infection induces endoplasmic reticulum (ER) membrane rearrangements to generate a compartment for replication of the viral genome and assembly of viral particles. Using quantitative mass spectrometry, we identified several ESCRT (endosomal sorting complex required for transport) proteins that are recruited to sites of virus replication on the ER. Systematic small interfering RNA (siRNA) screening revealed that release of both dengue virus and Japanese encephalitis virus was dramatically decreased by single depletion of TSG101 or co-depletion of specific combinations of ESCRT-III proteins, resulting in ≥1,000-fold titer reductions. By contrast, release was unaffected by depletion of some core ESCRTs, including VPS4. Reintroduction of ESCRT proteins to siRNA-depleted cells revealed interactions among ESCRT proteins that are crucial for flavivirus budding. Electron-microscopy studies revealed that the CHMP2 and CHMP4 proteins function directly in membrane deformation at the ER. Thus, a unique and specific subset of ESCRT contributes to ER membrane biogenesis during flavivirus infection.

INTRODUCTION

Flaviviruses are members of the single-stranded, positive-sense RNA (ssRNA(+)) viruses. This family of viruses includes dengue virus (DENV) and Japanese encephalitis virus (JEV), which cause large epidemics and tens of thousands of deaths annually in many parts of the world (Gould and Solomon, 2008). Virus assembly is a highly conserved process in viral life cycles, and, therefore, it represents a promising target for broad-spectrum antiviral therapies. For many enveloped viruses, viral particle formation requires recruitment of machinery normally involved in two analogous cellular membrane fission events, formation of small vesicles into multivesicular bodies (MVBs) and abscission during cytokinesis (McCullough et al., 2013). This machinery, whose components are called the endosomal sorting complex required for transport (ESCRT), functions on the plasma membrane or endosome to induce deformation of membranes away from the cytoplasm, and, ultimately, it pinches off of viral particles from the cell membrane (Hurley and Hanson, 2010).

Flaviviruses are replicated and assembled at the endoplasmic reticulum (ER), concomitant with dramatic rearrangement of membrane structure, to generate a specific compartment generally referred to as the viral replication organelle (Welsch et al., 2009). During viral genome replication, viruses produce small compartments in the ER lumen associated with the formation of vesicles whose outer membranes are connected to the ER via a neck-like structure. The newly synthesized genome is assembled on the ER membrane and buds into the ER lumen (Chatel-Chaix and Bartenschlager, 2014). This budding process is driven by the viral structural proteins prM and E. Recent studies revealed that NS2A and NS3, viral non-structural proteins, also play essential roles in this process (Carpp et al., 2011; Chiou et al., 2003), although their mechanistic roles have not been elucidated.

The ESCRT pathway is conserved in all eukaryotes and consists of several distinct heteromeric complexes that are sequentially recruited to sites of membrane deformation. ESCRT-0 recognizes ubiquitylated cargo; ESCRT-I, ESCRT-II, and ALIX play roles in the concentration of cargoes and deformation of membranes; and ESCRT-III constitutes the fission machinery. Mammalian cells express 12 ESCRT-III proteins, designated charged MVB protein (CHMP), which form at least two subcomplexes consisting of the CHMP6–CHMP4 family (A–C) and the CHMP3–CHMP2 family