Viruses Customize Autophagy Protein for Efficient Viral Entry

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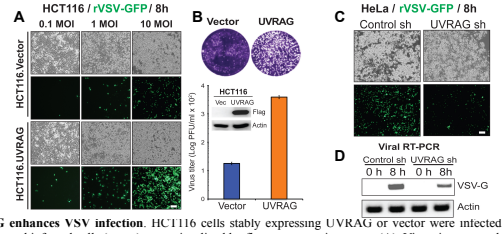
ABSTRACT

Negative-strand RNA viruses are highly pathogenic and cause many severe diseases in humans and animals. These viruses generally use existing cellular pathways to enter cells, which involves intensive interaction with the endomembrane network, offering the endocytic pathway as an attractive scheme for therapeutic intervention. The molecular mechanisms governing virus entry remain incompletely understood. We found that UVRAG, well-known for regulating autophagy and intracellular trafficking, is a critical factor for virus entry through combinatorial interactions with a tether and endosomal SNAREs. UVRAG mediates viral endocytic transport and membrane penetration through interactions with the class C Vps complex and endosomal Q-SNAREs, leading to the assembly of a fusogenic *trans*-SNARE complex involving VAMP8, but not VAMP7. Indeed, UVRAG stimulates VAMP8 translocation to virus-bearing endosomes. Inhibition of VAMP8, but not VAMP7, reduces viral entry. Understanding the mechanism that allows the virus to interact with late endocytic organelles could identify the specific set of proteins that have a role in virus entry, which help us to design specific therapeutic agents against virus entries.

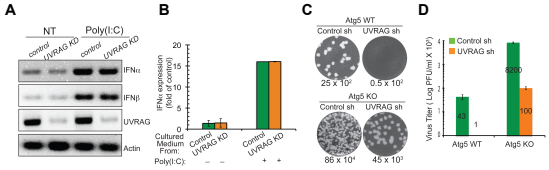
1 UVRAG is required for efficient viral infection



(A-B) UVRAG enhances VSV infection. HCT116 cells stably expressing UVRAG or vector were infected with rVSV-GFP for 8 hr, and infected cells (green) were visualized by fluorescence microscopy (A). Virus titers were determined by plaque assay (B).

(C-D) UVRAG deficiency restricts VSV infection. Control- or UVRAG-knockdown HeLa cells were challenged with VSV. Infection was assessed by IF for GFP (C) and viral RNA was quantified by RT-PCR.

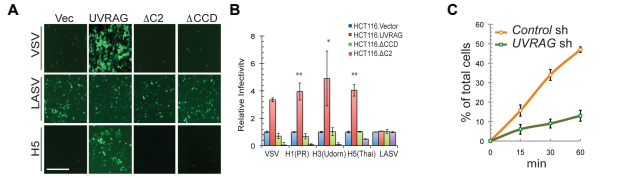
2 Effect of UVRAG is independent of interferon and autophagy



(A-B) *UVRAG* knockdown has minimal effect on IFN production. HeLa cells with control or UVRAG knockdown were treated with poly(I:C) and the induction of IFNα and IFNβ was determined by RT-qPCR (A) and quantified (B).

(C-D)*UVRAG* knockdown suppresses VSV infection in autophagy-deficient cells. *Atg5*$^{+/+}$ and *Atg5*$^{-/-}$ immortalized MEF cells were transfected with either control shRNA or UVRAG-specific shRNA. VSV infection of MEF cells was determined by plaque assay (C) and the resultant virus titers were quantified in (D).

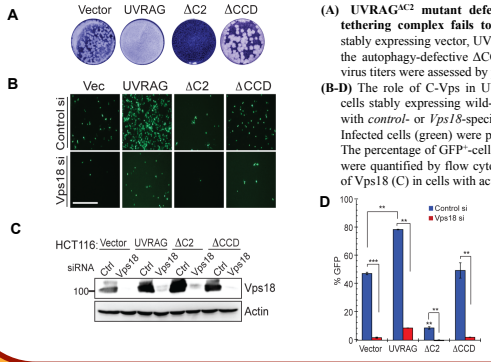
3 UVRAG mediates viral entry by promoting endocytic transport of virions



(A-B) UVRAG knockdown impairs virus entry of VSV and IAV. HeLa cells were transfected with control- or UVRAG-specific shRNA, then infected with MLV-GFP pseudotyped with the indicated envelope protein (VSV, IAV H1N1 [PR8], IAV H3N1 [Udom], IAV H5N1 [Thai], LASV, or LCMV). Representative images of viral infection (green) were shown (**A**). Viral entry is expressed as mean GFP fluorescence relative to control cells, determined by flow cytometry (**B**).

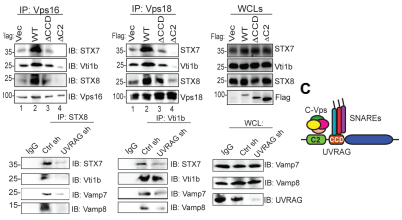
(C) UVRAG is required for proper delivery of virion to late endosomes. UVRAG depletion inhibits late endosomal transport of VSV. HeLa cells were transfected with control- or UVRAG-shRNA, infected with DiI-labeled VSV at 4°C. The temperature was then shifted to 37°C to allow endocytosis. The number of cells containing DiI-dequenching signals was counted at the indicated time and is expressed as a percentage of the total cell numbers.

4 C/Vps interaction is required for UVRAG-mediated virus entry



(A) UVRAG$^{\Delta C2}$ mutant defective in binding the C/Vps endosome tethering complex fails to support VSV infection. HCT116 cells stably expressing wild-type or mutant UVRAG were transfected with *control*- or *Vps18*-specific siRNA, then infected with rVSV-GFP. Infected cells (green) were processed for fluorescence microscopy (B). The percentage of GFP$^+$-cells in control- or Vps18-siRNA-treated cells were quantified by flow cytometry (D). Western blot shows the levels of Vps18 (C) in cells with actin serving as a loading control.

5 UVRAG Enhances C-Vps Interaction with SNAREs and *trans*-SNARE Assembly

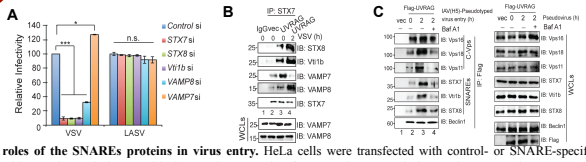


(A)UVRAG promotes the complex assembly of C-Vps and SNAREs. 293T were transfected with flag-tagged wild-type and mutant UVRAG proteins, followed by IP with anti-Vps16 (left) or anti-Vps18 (right), then IB with the indicated antibodies. Right panel shows transfected and endogenous protein expression.

(B)UVRAG is required for the SNAREs assembly. 293T cells were transfected with control shRNA or UVRAG shRNA. WCLs were IP with anti-STX8 or anti-Vti1b followed by IB with the indicated antibodies. Right panel shows endogenous protein expressions.

(C)Schematic representation of the UVRAG-C-Vps-SNARE super-complex.

6 Virus Entry Stimulates a UVRAG/C-Vps/SNAREs Complex Assembly



(A)Distinct roles of the SNAREs proteins in virus entry. HeLa cells were transfected with control- or SNARE-specific siRNA, then infected with pseudotyped viruses with the envelope protein of VSV and LASV. Viral entry is expressed as mean GFP fluorescence relative to *control* sh-treated cells

(B)The R-SNAREs VAMP8, but not VAMP7, is involved in UVRAG-mediated SNARE complex assembly upon virus entry. 293T were transfected with vector or Flag-UVRAG, then mock-infected, or infected with VSV. WCLs were IP with anti-STX7, followed by IB with the indicated antibodies. Note that the VAMP8-STX7 interaction was drastically induced by VSV.

(C) IAV-H5-mediated entry process promotes the complex formation of UVRAG, C-Vps, and Q-SNAREs. 293T transfected with vector or Flag-UVRAG, were infected with pseudovirus of IAV w/o Bafilomycin A_1. WCLs were IP with anti-UVRAG, followed by IB with the indicated antibodies against C-Vps subunits and SNARE proteins.

CONCLUSION

Negative-strand RNA viruses are highly pathogenic and cause many severe diseases in humans and animals. These viruses generally use existing cellular pathways to enter cells, which involves intensive interaction with the endomembrane network, offering the endocytic pathway as an attractive scheme for therapeutic intervention. The molecular mechanisms governing virus entry remain incompletely understood. We found that UVRAG, well-known for regulating autophagy and intracellular trafficking, is a critical factor for virus entry through combinatorial interactions with a tether and endosomal SNAREs. Understanding the mechanism that allows the virus to interact with late endocytic organelles could identify the specific set of proteins that have a role in virus entry, which help us to design specific therapeutic agents against endocytic virus entries.

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