Autophagy 8:7, 1136–1137; July 2012; © 2012 Landes Bioscience

Autophagy and RNA virus interactomes reveal IRGM as a common target

Isabel Pombo Grégoire,[1,2,3] Chantal Rabourdin-Combe[1,2,3] and Mathias Faure[1,2,3,*]

[1]Université de Lyon; Lyon, France; INSERM; [2]U851; Lyon, France; [3]UMS3444/US8; Université Lyon 1; Lyon, France

Several intracellular pathogens have the ability to avoid or exploit the otherwise destructive process of autophagy. RNA viruses are constantly confronted with cellular autophagy, and several of them hijack autophagy during the infectious cycle to improve their own replication. Nevertheless, our knowledge of viral molecular strategies used to manipulate autophagy remains limited. Our study allowed the identification of molecular interactions between 44 autophagy-associated proteins and 83 viral proteins belonging to five different RNA virus families. This interactome revealed that the autophagy network machinery is highly targeted by RNA viruses. Interestingly, whereas some autophagy-associated proteins are targeted by only one RNA virus family, others are recurrent targets of several families. Among them, we found IRGM as the most targeted autophagy-associated protein. Downregulation of IRGM expression prevents autophagy induction by measles virus, HCV and HIV-1, and compromises viral replication. Our work combined interactomic and analytical approaches to identify potential pathogen virulence factors targeting autophagy.

Keywords: autophagy, network, interactome, RNA viruses, measles virus

Submitted: 04/05/12

Revised: 04/06/12

Accepted: 04/10/12

http://dx.doi.org/10.4161/auto.20339

*Correspondence to: Mathias Faure; Email: mathias.faure@inserm.fr

Punctum to: Grégoire IP, Richetta C, Meyniel-Schicklin L, Borel S, Pradezynski F, Diaz O, et al. IRGM is a common target of RNA viruses that subvert the autophagy network. PLoS Pathog 2011; 7:e1002422; PMID:22174682; http://dx.doi.org/10.1371/journal.ppat.1002422

Autophagy is a highly conserved, self-degradative pathway for the clearance and recycling of cytoplasmic contents. This ubiquitous cell intrinsic process can be used as a defense mechanism against intracellular pathogens. However, host-cell/pathogen co-evolution has selected numerous micoorganisms able to avoid autophagy or even usurp this mechanism to their own benefit. Among them, several RNA viruses that include species of major concern in public health (e.g., HCV, HIV-1, influenza A, Dengue virus, Chikungunya virus) were shown to subvert autophagy, nevertheless few viral molecular adaptations to host autophagy have yet been identified. We previously reported that attenuated strains of measles virus (MeV, an RNA virus) induce autophagy through the engagement of its cellular receptor CD46. Furthermore, we found that MeV hijacks autophagy to improve its infectivity.

RNA viruses encode a small number of proteins. Nevertheless, to establish a productive infection, multiple cellular molecular processes have to be controlled to avoid viral destruction and allow efficient replication. To identify which components of the autophagy machinery RNA viruses target, we used a global approach based on the confrontation of the restricted autophagy protein network with RNA virus proteins. Using a yeast two-hybrid approach and literature curation, we determined the interactome between 44 autophagy-associated proteins and 83 viral proteins belonging to several RNA virus families (*Paramyxoviridae*, *Orthomyxoviridae*, *Flaviviridae*, *Togaviridae*, *Coronaviridae*, *Bornaviridae* and *Retroviridae*). The autophagy-associated proteins were selected on the basis of reports describing them as modulators of the autophagy process, either by overexpression or downmodulation. Our aim being to identify viral targets particularly involved in autophagy modulation (with no or limited modulation of other cellular processes), we excluded from our selected list several autophagy-associated proteins that, despite their crucial involvement in autophagy, are broad signaling regulators of several other cellular pathways.

We found that RNA viruses target more than 35% of the autophagy-associated

proteins (17/44 proteins). This suggests that autophagy is a highly and significantly targeted cellular process. We further determined how autophagy proteins interact with each other and identified a highly interconnected network with 150 intranetwork interactions. Bioinformatic analysis of the protein-protein interactions allowed us to highlight the relative importance of each autophagy-associated protein within the autophagy network, when compared with the whole human proteome. This analysis suggested that RNA viruses mostly target autophagy-associated proteins that are essential (central) to autophagy (14/17 proteins). Interestingly, some autophagy-associated proteins are targeted only by one RNA virus family (9/17 proteins). In contrast, others are commonly targeted by several RNA virus families (8/17 proteins). These observations might reflect virus-specific and virus-common strategies to manipulate autophagy, respectively.

We then focused our study on one particular autophagy-associated protein, immunity-associated GTPase family M (IRGM), for several reasons. First, IRGM was found to be targeted by five different RNA virus families, including MeV, HCV, HIV-1, influenza A and Chikungunya virus, all known to manipulate autophagy. Second, the IRGM-described function was particularly related to autophagy. Third, IRGM was previously reported to play an autophagy-dependent anti-bacterial function, but no report defined a role for this protein during viral infection. Fourth, how IRGM contributes to autophagy was unknown; in particular, no protein partners for IRGM had yet been reported. We found in our study that IRGM interacts with four autophagy-associated proteins, (ATG5, ATG10, MAP1LC3C and SH3GLB1). Together, these results suggested that IRGM might be particularly dedicated to autophagy, and that selected RNA viruses might have evolved molecular strategies to modulate autophagy by targeting this cellular protein. Interestingly, the identified IRGM molecular partners are all involved in the initiation/elongation phases suggesting that IRGM would modulate the initial steps of autophagy.

The up-to-now unique function of IRGM in autophagy led us to hypothesize that viruses modulate autophagy through IRGM targeting. We found that IRGM downregulation prevents MeV-, HCV- and HIV-1-induced autophagy. Additionally, the absence of IRGM limits MeV, HCV and HIV-1 viral production. Importantly, we found that IRGM colocalizes and interacts with MeV-C, HCV-NS3 and HIV-NEF proteins in cotransfected mammalian cells. Finally, we found that the expression of MeV-C, HCV-NS3 or HIV-NEF per se is sufficient to modulate autophagosome formation and that IRGM is required for this process. Thus, our results suggest that through the expression of a unique protein, viruses might induce and manipulate autophagy to potentiate their replication. An alternative possibility would be that IRGM plays a role in viral sensing leading to an autophagy-mediated anti-viral response, and that additional autophagy-associated proteins might be targeted by viruses, which exploit autophagy to their own benefit. Additional studies are required to fully decipher the molecular mechanism involving IRGM during virus-induced autophagy.

Our work described IRGM as a key and common positive modulator of autophagy usurped by several RNA viruses to manipulate this cellular process, and identified the viral proteins involved in this subversion. In addition, our global approach offers to the scientific community a broad sample of data for the study of host autophagy-RNA virus relationships and beyond. Understanding the mechanisms underlying the ability of certain pathogens to escape autophagy is crucial if one aims to manipulate this cellular function in order to prevent or treat infectious diseases.

In Memoriam Chantal Rabourdin-Combe

We dedicate this puncta to Chantal Rabourdin-Combe who inspired this work and far beyond.