At the crossroads of autophagy and infection: Noncanonical roles for ATG proteins in viral replication

Tina Solvik[1,2,3] and Jayanta Debnath[1,2]

[1]Department of Pathology, [2]Helen Diller Family Comprehensive Cancer Center, and [3]Biomedical Sciences Graduate Program, University of California, San Francisco, San Francisco, CA 94143

Autophagy-related (ATG) proteins have increasingly demonstrated functions other than cellular self-eating. In this issue, Mauthe et al. (2016. J. Cell Biol. http://dx.doi.org/10.1083/jcb.201602046) conduct an unbiased RNA interference screen of the ATG proteome to reveal numerous noncanonical roles for ATG proteins during viral infection.

Macroautophagy (autophagy) involves the formation of a double-membrane organelle called the autophagosome, which sequesters cytoplasmic components that are degraded upon its fusion with the lysosome (Stolz et al., 2014; Kaur and Debnath, 2015). Studies in yeast have identified >30 autophagy-related (ATG) proteins, many of which have identified mammalian orthologues. The canonical process of autophagy begins with initiation at the phagophore assembly site, mediated by the UNC51-like kinase (ULK) complex, which is composed of the ATG proteins ULK1/2, ATG13, FIP200, and ATG101. The ULK complex supports the activation of a class III phosphatidylinositol 3-kinase complex consisting of Beclin 1 (ATG6), ATG14, and VPS34 to produce phosphatidylinositol triphosphate, which serves as the initial membrane mark recognized by early autophagic effector proteins. Phagophore membrane expansion is mediated by the two ubiquitin-like conjugation systems involving multiple ATG proteins that ultimately lead to the lipidation of microtubule-associated protein 1 light chain 3 (LC3), the mammalian orthologue of ATG8. LC3 is recognized by autophagy cargo receptors that promote the selective capture and engulfment of proteins, organelles, or microbes. The closed autophagosome is then trafficked to and fuses with the lysosome to degrade its cargo (Stolz et al., 2014; Kaur and Debnath, 2015). ATG proteins traditionally have been studied for their roles in autophagosome formation and maturation, but we have recently begun to appreciate that they may have pleiotropic roles beyond autophagy (Bestebroer et al., 2013). In this issue, through a comprehensive functional screen for ATG proteins in the control of viral replication, Mauthe et al. highlight the importance of the nontraditional functions of ATG proteins in the host viral response.

In a tour-de-force study examining multiple models of viral infection, Mauthe et al. (2016) interrogated the individual roles of ATG proteins in the replication of herpes simplex virus type 1, mouse hepatitis virus (MHV), vaccinia virus (VaV), semliki forest virus, encephalomyocarditis virus, and influenza A virus. The authors generated a curated siRNA library to target all ATG proteins individually as well as functionally redundant groups concurrently to evaluate how they promote the replication of these six viruses in two model cell lines (HeLa and U2OS). Indeed, their screen reveals that unconventional functions of ATG proteins are widespread; overall, 16 out of the 44 tested ATG proteins (36%) appear to have unconventional functions. Rarely does knockdown of individual ATG genes result in concordant effects on viral replication, arguing against a general role for autophagy. Moreover, the authors fail to identify any single ATG protein that regulates viral replication in all of the tested viruses; rather, specific ATG genes influenced the replication of each virus. Remarkably, most of the ATG proteins show a beneficial role for VaV replication in U2OS cells yet negatively impact VaV replication in HeLa cells. Overall, the effect of ATG loss of function on viral replication is exquisitely ATG specific, virus specific, and cell line specific.

To demonstrate functions of ATG proteins independent of autophagy, the authors scrutinized ATG13 and FIP200, two ULK complex proteins that initiate formation of the autophagosome, and found that these proteins exert effects on viral replication distinct from the effects of other ULK complex components. Mauthe et al. (2016) demonstrate that depletion of ATG13 and FIP200 reduces the replication of two picornaviruses, encephalomyocarditis virus and coxsackievirus (CV) B3, whereas the other ULK complex components promote their replication. Furthermore, a principal upstream regulator of ULK complex activity, mammalian target of rapamycin complex 1, showed no changes in activity during infection or knockdown of the ULK complex components. Altogether, this evidence indicates that ATG13 and FIP200 act independently of the ULK complex to modify picornaviral replication outside the context of autophagy.

Although the precise mechanism of this unconventional function of ATG13 and FIP200 remains to be elucidated, Mauthe et al. (2016) uncovered that these proteins control viral replication as opposed to viral entry or viral translation. However, ATG13 and FIP200 do not interact with any CV proteins or affect the morphology of CV replication structures, suggesting

Correspondence to Jayanta Debnath: jayanta.debnath@ucsf.edu

The Rockefeller University Press $30.00
J. Cell Biol. Vol. 214 No. 5 503–505
www.jcb.org/cgi/doi/10.1083/jcb.201608032

the proteins have an indirect effect on viral replication. RNA sequencing analysis demonstrates that ATG13 and FIP200 affect genes predominantly involved in transcriptional regulation during CV infection. Future studies expanding on these results are needed to dissect the exact mechanism by which ATG13 and FIP200 reduce picornaviral replication.

Autophagy has inexorably been linked to the host cellular response to diverse intracellular pathogens. Indeed, autophagy frequently serves as an innate mechanism that sequesters or clears offending pathogens from the host cell, termed xenophagy. In response to infection, pathogen- and damage-associated molecular patterns stimulate pattern recognition receptors, which then activate ATG proteins to induce autophagy or promote recognition of the microbe by the LC3-positive autophagosome (Deretic et al., 2015). Nevertheless, the autophagy trafficking process can be subverted by pathogens that have adapted to avoid or use autophagy to their own benefit (Deretic and Levine, 2009). One example is poliovirus, which utilizes LC3 to promote its nonlytic viral release, potentially by the fusion of LC3-positive vesicles containing poliovirus with the plasma membrane (Bird et al., 2014).

Interestingly, this last example broaches how ATG proteins, either as individual proteins or in groups, may enable unconventional functions distinct from the canonical process of autophagy. This can include entirely novel functions or ATG processes that use only a subset of the core components (Bestebroer et al., 2013). Before Mauthe et al. (2016), a comprehensive, unbiased analysis of the entire ATG proteome to uncover the autophagy-independent functions of ATG proteins during infection had not been undertaken. Nevertheless, other studies have highlighted some of the emerging noncanonical roles of ATG proteins during microbial pathogenesis. A well characterized example is LC3-associated phagocytosis (LAP), in which a select group of ATG proteins promotes maturation and degradation of the phagosome. Upon toll-like receptor-triggered phagocytosis, Beclin 1 is recruited to the phagosome, where it induces production of phosphatidylinositol triphosphate and localization of LC3, causing fusion with the lysosome (Sanjuan et al., 2007). Although LAP requires several ATG proteins—Beclin 1, LC3, ATG5, and ATG7, among others—it is mechanistically distinct from autophagy as it does not use the ULK complex that initiates canonical autophagy. In addition, LAP utilizes proteins such as Rubicon, which is inhibitory to autophagy, further demonstrating deviation from the conventional functions of ATG proteins (Martinez et al., 2015). LAP serves as a host defense system that targets several pathogens, including *Salmonella typhimurium* and *Aspergillus fumigatus* (Huang et al., 2009; Martinez et al., 2015).

In other cases, the growing list of noncanonical functions of ATG proteins has led the field to reevaluate a general role of the autophagy pathway in certain aspects of microbial pathogenesis. First, the autophagy pathway was proposed to defend against *Mycobacterium tuberculosis* infection because induction of autophagy by starvation or treatment with rapamycin reduces *M. tuberculosis* viability and because myeloid-specific deletion of ATG5 results in increased inflammation and bacterial load in the lungs (Castillo et al., 2012; Watson et al., 2012). Although this result originally assumed a protective function of autophagy, recent work demonstrates that ATG5 is unique in its ability to restrict *M. tuberculosis* infection. In stark contrast to ATG5, the genetic deletion of other essential ATG genes (ATG3, ATG7, ATG12, ATG14L, and ATG16L) has no effect on *M. tuberculosis* infection in vivo. Instead, ATG5 prevents accumulation of polymorphonuclear cells in the lungs, thereby controlling inflammation and *M. tuberculosis* replication (Kimmey et al., 2015).

During *Brucella abortus* infection, multiple ATG proteins implicated in the early steps of autophagosome initiation, such as ULK1 and Beclin 1, are involved in the formation of a *B. abortus*–containing vacuole (BCV) with autophagic features (aBCV), which promotes bacterial proliferation. Double-membrane crescents reminiscent of the autophagosome engulf BCVs late in infection but do not degrade or clear the bacteria. Instead, ULK1, Beclin 1, and class III phosphatidylinositol 3-kinase activity are all required for the conversion of BCVs into aBCVs. However, the ATG conjugation pathways involved in autophagosome elongation play no role in aBCV formation. Furthermore, these aBCVs facilitate bacterial release and infection of neighboring cells, demonstrating that very specific components of autophagosome initiation promote *B. abortus* infection (Starr et al., 2012).

Moreover, the lipidated form of LC3 (LC3-II) is essential for autophagosome formation and crucially interacts with cytoplasmic cargo to be degraded. However, during MHV infection, unlipidated LC3 (LC3-I) promotes viral replication in double-membrane vesicles (DMVs). The similarity of DMVs to autophagosomes prompted speculation that classical autophagy controlled MHV infection. However, ATG5 does not affect infection, and only LC3-I colocalizes with MHV DMVs, suggesting that canonical autophagy is not involved (Zhao et al., 2007). Further investigation revealed that two proteins in the ER-associated degradation pathway interact with LC3-I and MHV replication translation complexes, that MHV DMVs are of ER origin, and that LC3-I, but not LC3-II, is required for MHV replication (Reggiori et al., 2010). Remarkably, these LC3-I–positive DMVs represent another LC3-presenting membrane distinct from the canonical double-membrane autophagosome as well as the LC3-II–positive single-membrane phagosome in LAP.

Lastly, during mouse norovirus (MNV) infection, IFN-γ–activated phagocytes use ATG5-ATG12/ATG16L1 to inhibit formation of the viral replication complex, thus restricting infection. This ATG complex typically promotes LC3 lipidation, resulting in expansion of the autophagosome. Based on the requirement for ATG5 in IFN-γ–mediated inhibition of MNV replication, autophagy was initially suggested as the mechanism of host defense. However, IFN-γ has no effect on autophagy in phagocytes, and inducing or inhibiting autophagy does not alter MNV replication. Instead, only ATG5-ATG12/ATG16L1 is required for MNV replication inhibition, and ATG16L1 localizes to the MNV replication complex. ATG5-ATG12/ATG16L1 is required for IFN-γ–mediated suppression of MNV polymerase expression and, therefore, replication as a whole, an example of multiple ATG proteins functioning to inhibit viral infection (Hwang et al., 2012).

Ultimately, the discovery of widespread autophagy-independent functions of ATG proteins marks a significant turning point as well as reinforces an important caveat for the field. Going forward, researchers must use caution when attributing a phenotype to canonical autophagy based on the genetic analysis of a single ATG protein. The experiments in Mauthe et al. (2016) poignantly illustrate the need to functionally test multiple ATG proteins when characterizing a phenotype to ascertain a general role for autophagy as opposed to a distinct function mediated by individual ATG proteins.

Acknowledgments

J. Debnath is supported by the National Institutes of Health (CA126792, CA188404, and CA201849), U.S. Department of Defense Breast Cancer Research Program (W81XWH-11-1-0130), and Samuel Waxman Cancer Research Foundation.

The authors declare no competing financial interests.

Submitted: 8 August 2016
Accepted: 8 August 2016

References

Bestebroer, J., P. V'kovski, M. Mauthe, and F. Reggiori. 2013. Hidden behind autophagy: the unconventional roles of ATG proteins. *Traffic.* 14:1029–1041. http://dx.doi.org/10.1111/tra.12091

Bird, S.W., N.D. Maynard, M.W. Covert, and K. Kirkegaard. 2014. Nonlytic viral spread enhanced by autophagy components. *Proc. Natl. Acad. Sci. USA.* 111:13081–13086. http://dx.doi.org/10.1073/pnas.1401437111

Castillo, E.F., A. Dekonenko, J. Arko-Mensah, M.A. Mandell, N. Dupont, S. Jiang, M. Delgado-Vargas, G.S. Timmins, D. Bhattacharya, H. Yang, et al. 2012. Autophagy protects against active tuberculosis by suppressing bacterial burden and inflammation. *Proc. Natl. Acad. Sci. USA.* 109:E3168–E3176. http://dx.doi.org/10.1073/pnas.1210500109

Deretic, V., and B. Levine. 2009. Autophagy, immunity, and microbial adaptations. *Cell Host Microbe.* 5:527–549. http://dx.doi.org/10.1016/j.chom.2009.05.016

Deretic, V., T. Kimura, G. Timmins, P. Moseley, S. Chauhan, and M. Mandell. 2015. Immunologic manifestations of autophagy. *J. Clin. Invest.* 125:75–84. http://dx.doi.org/10.1172/JCI73945

Huang, J., V. Canadien, G.Y. Lam, B.E. Steinberg, M.C. Dinauer, M.A. Magalhaes, M. Glogauer, S. Grinstein, and J.H. Brumell. 2009. Activation of antibacterial autophagy by NADPH oxidases. *Proc. Natl. Acad. Sci. USA.* 106:6226–6231. http://dx.doi.org/10.1073/pnas.0811045106

Hwang, S., N.S. Maloney, M.W. Bruinsma, G. Goel, E. Duan, L. Zhang, B. Shrestha, M.S. Diamond, A. Dani, S.V. Sosnovtsev, et al. 2012. Nondegradative role of Atg5-Atg12/Atg16L1 autophagy protein complex in antiviral activity of interferon gamma. *Cell Host Microbe.* 11:397–409. http://dx.doi.org/10.1016/j.chom.2012.03.002

Kaur, J., and J. Debnath. 2015. Autophagy at the crossroads of catabolism and anabolism. *Nat. Rev. Mol. Cell Biol.* 16:461–472. http://dx.doi.org/10.1038/nrm4024

Kimmey, J.M., J.P. Huynh, L.A. Weiss, S. Park, A. Kambal, J. Debnath, H.W. Virgin, and C.L. Stallings. 2015. Unique role for ATG5 in neutrophil-mediated immunopathology during *M. tuberculosis* infection. *Nature.* 528:565–569. http://dx.doi.org/10.1038/nature16451

Martinez, J., R.K. Malireddi, Q. Lu, L.D. Cunha, S. Pelletier, S. Gingras, R. Orchard, J.L. Guan, H. Tan, J. Peng, et al. 2015. Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. *Nat. Cell Biol.* 17:893–906. http://dx.doi.org/10.1038/ncb3192

Mauthe, M., M. Langereis, J. Jung, X. Zhou, A. Jones, W. Omta, S.A. Tooze, B. Stork, S.R. Paludan, T. Ahola, et al. 2016. An siRNA screen for ATG protein depletion reveals the extent of the unconventional functions of the autophagy proteome in virus replication. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201602046

Reggiori, F., I. Monastyrska, M.H. Verheije, T. Calì, M. Ulasli, S. Bianchi, R. Bernasconi, C.A. de Haan, and M. Molinari. 2010. Coronaviruses hijack the LC3-I-positive EDEMosomes, ER-derived vesicles exporting short-lived ERAD regulators, for replication. *Cell Host Microbe.* 7:500–508. http://dx.doi.org/10.1016/j.chom.2010.05.013

Sanjuan, M.A., C.P. Dillon, S.W. Tait, S. Moshiach, F. Dorsey, S. Connell, M. Komatsu, K. Tanaka, J.L. Cleveland, S. Withoff, and D.R. Green. 2007. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature.* 450:1253–1257. http://dx.doi.org/10.1038/nature06421

Starr, T., R. Child, T.D. Wehrly, B. Hansen, S. Hwang, C. López-Otin, H.W. Virgin, and J. Celli. 2012. Selective subversion of autophagy complexes facilitates completion of the Brucella intracellular cycle. *Cell Host Microbe.* 11:33–45. http://dx.doi.org/10.1016/j.chom.2011.12.002

Stolz, A., A. Ernst, and I. Dikic. 2014. Cargo recognition and trafficking in selective autophagy. *Nat. Cell Biol.* 16:495–501. http://dx.doi.org/10.1038/ncb2979

Watson, R.O., P.S. Manzanillo, and J.S. Cox. 2012. Extracellular *M. tuberculosis* DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. *Cell.* 150:803–815. http://dx.doi.org/10.1016/j.cell.2012.06.040

Zhao, Z., L.B. Thackray, B.C. Miller, T.M. Lynn, M.M. Becker, E. Ward, N.N. Mizushima, M.R. Denison, and H.W. Virgin IV. 2007. Coronavirus replication does not require the autophagy gene ATG5. *Autophagy.* 3:581–585. http://dx.doi.org/10.4161/auto.4782