Role of autophagy in Drosophila innate immunity

H Nagai, T Yano, S Kurata

Graduate School of Pharmaceutical Sciences, Tohoku University, Japan

To the Editor

Autophagy is a well-conserved intracellular degradation system in which cytoplasmic components and organelles are recycled by double-membraned autophagosomes that engulf them and eventually fuse with lysosomes (Levin et al., 2011). Because most of the genes involved in autophagy are conserved from yeast to invertebrates and mammals, and genetic manipulations can easily be performed in Drosophila in vivo, Drosophila is an ideal model system for studying the function of autophagy in whole animals. Studies of autophagy-related gene mutants revealed the importance of autophagic functions in larval midgut cell death during morphogenesis (Denton et al., 2009), and hematopoiesis in larvae (Shravage et al., 2013). In addition to its essential functions in development, emerging evidence indicates that autophagy functions against invasion of host cells by pathogens such as intracellular bacteria, viruses, and protozoa (Yano and Kurata, 2011). Here, we summarize and provide insight into the functions of autophagy in innate immunity in invertebrates.

The first evidence that autophagy is crucial for protection against intracellular bacterial growth came from a study of Group A Streptococcus, which invades mammalian culture cells (Nakagawa et al., 2004). Many subsequent studies demonstrated that intracellular bacteria, such as Salmonella typhimurium, Listeria monocytogenes, and Mycobacterium tuberculosis are eliminated by autophagosomes, indicating the critical role of autophagy in innate immune responses against such bacteria (Huang and Brumell, 2014). Because studies were performed in cultured cells and autophagy-knockout animals are in most cases lethal, the critical role of autophagy in vivo was first demonstrated in Drosophila by tissue-specific autophagy knockdown (Yano et al., 2008). Listeria utilizes the host endocytic pathway to enter Drosophila hemocytes, the hematopoietic cells that function in innate immunity. When Listeria infects the body cavity, they invade the hemocytes because of their high phagocytic ability, but are quickly engulfed by autophagosomes for elimination. Flies in which the autophagy gene atg5 is specifically knocked down in hemocytes are susceptible to Listeria infection, suggesting that autophagy is important for their survival against infection by Listeria (Yano et al., 2008). The importance of autophagy in protecting against invasive bacteria has also been demonstrated in another insect, Tenebrio molitor, in which knockdown of the autophagy genes Atg3 or Atg5 leads to susceptibility to Listeria in vivo (Tindwa et al., 2015).

Although basal levels of autophagy function in the basically non-selective turnover of cytosolic molecules, autophagy is selectively induced for the degradation of aggregate proteins, damaged mitochondria, or invading bacteria (Mizushima and Komatsu, 2011). Autophagosomes that surround the bacteria are spatially regulated, and autophagy is selectively induced upon the recognition of bacteria by the pattern recognition receptors (PRRs) of the host. In Drosophila cells, intracellular PRRs, such as peptidoglycan-recognition protein (PGRP)-LE, recognize and bind to peptidoglycans of the bacterial cell walls, and this recognition is necessary for autophagy induction (Yano et al., 2008). Compared to Listeria infection of Drosophila cells, which is largely dependent on PGRP-LE for induction, mammalian cells have similar, but more complicated systems: several triggers are involved in the induction of autophagy, including recognition by PRRs such as NOD2, ubiquitination of proteins around the bacteria, and destruction of the host endosomes used by the bacteria to enter the host cells (Gomes and Dikic, 2014). Mammalian cells likely adapt to the strategies bacteria use to escape from autophagic elimination.

Ubiquitin may be one sign of autophagic engulfment (Boyle and Randow, 2013). Ubiquitin is often observed around bacteria invading the cytosol by breaking the endosomal membrane. In mammalian cells, the adaptor proteins p62, NDP52, and optineurin recognize the ubiquitinated bacteria, and recruit autophagosomes to the bacteria by binding to LC3/Atg8 protein located on the autophagosomes (Huang and Brumell, 2014). Although ubiquitinated proteins clearly colocalize with the invading bacteria, it is unknown how the ubiquitin-modified proteins are identified. The E3 ligase LRSAM1 is required for ubiquitination in mammalian cells upon Salmonella infection, and the enzyme is essential for colocalization of the adaptor...
proteins and bacteria (Huett et al., 2012). The E3 ligase Parkin is also involved in the induction of ubiquitin-dependent autophagy upon invasion by M. tuberculosis (Manzanillo et al., 2013). Parkin (Park2 in mammals) is an essential E3 ligase for autophagic clearance of damaged mitochondria (mitophagy), but it also functions to induce autophagy upon Mycobacterium infection (xenophagy), which limits bacterial replication. Parkin is essential for mitochondrial maintenance in Drosophila as well as in mammals, and its xenophagic function is also conserved. Flies with a mutant parkin gene are susceptible to L. monocytogenes, S. typhimurium, and M. marinum infection (Manzanillo et al., 2013), indicating striking similarities between the mitophagy and xenophagy mechanisms.

In addition to its anti-bacterial functions, autophagy is important as an innate immune response against viruses in both mammalian cells and Drosophila. Vesicular stomatitis virus (VSV), a rhabdovirus that infects Drosophila S2 cells, rapidly replicates when autophagy is inhibited (Shelly et al., 2009). Moreover, Atg18 or Atg7 knockdown flies exhibit increased susceptibility to VSV, suggesting that autophagy is essential for fly survival against VSV infection. The induction of autophagy upon VSV infection is dependent on the viral glycoprotein VSV-G, and Toll-7, a Drosophila Toll-like receptor that interacts with VSV-G on the plasma membrane to trigger autophagy via a nutrient signaling pathway for autophagy induction, the PI3K-AKT-TOR pathway (Nakamoto et al., 2012). Extracellular signal-related kinase signaling has a role in the anti-RNA virus activity in Drosophila cells and fly intestine by coupling nutrient status to antiviral defense (Xu et al., 2013). The precise mechanism underlying how autophagy inhibits viral replication, however, remains to be elucidated.

The Drosophila p62 homologue ref(2)P is another candidate molecule that functions in the anti-viral response via autophagy. The ref(2)P gene is polymorphic, and some wild populations carrying restrictive alleles exhibit reduced multiplication rates of the sigma virus, a vertically transmitted rhabdovirus (Carré-Mlouka et al., 2007). In this case, it is not known whether the ref(2)P-dependent restriction of viral replication occurs via autophagy.

Autophagy has a critical role in anti-bacterial immunity not only in phagocytic immune cells, such as macrophages in mammals or hemocytes in flies, but also in epithelial cells. Autophagy in mouse intestinal epithelial cells (IECs) inhibits the growth of S. typhimurium, an intestinal pathogen that invades IECs to cause inflammation (Benjamin et al., 2013). Mice with IEC-specific knockout of Atg5 are unable to eliminate S. typhimurium, resulting in dissemination of the bacteria to other organs. Infection by S. typhimurium induces autophagy, especially at the apical side of IECs. This raises the possibility that the host quickly recognizes invading S. typhimurium to induce autophagy. The induction of autophagy against S. typhimurium is independent of NOD2, an intracellular pathogen recognition protein that induces xenophagy (Travassos et al., 2010), but rather depends on MyD88, a signaling molecule of the Toll pathway that leads to nuclear factor κB activation. How MyD88 contributes to autophagy induction in IECs, however, remains to be elucidated. Although in Drosophila less is known about the autophagic role against invasive bacteria in the intestine, the powerful genetic techniques available for Drosophila could provide new insight.

In addition to its role in immunity, autophagy is related to inflammation. Recent genome-wide association studies identified the autophagy-related genes Atg16L1, IRGM, and NOD2 as risk factors for Crohn’s disease, a chronic inflammatory bowel disease (Hampe et al., 2007; Rioux et al., 2007). The pathogenesis of this disease is also affected by environmental factors, such as commensal bacteria. The mechanism of inflammatory diseases caused by defective autophagy was studied in Atg16L1 gene knockout mice: overproduction of interleukin-1β, an inflammatory cytokine, in Atg16L1-knockout macrophages is stimulated by commensal bacteria such as Escherichia coli (Saltoh et al., 2008), whereas dysfunction of Paneth cells, immune cells that reside in intestinal crypts and secrete antimicrobial peptides and lysozymes, occurs in Atg16L1-hypomorphic mice and IEC-specific Atg16L1 knockout mice (Cadwell et al., 2008; Cadwell et al., 2010; Conway et al., 2013). Interestingly, this Paneth cell secretion defect is also dependent on enteric bacteria. Although these studies elucidated the importance of autophagy (genetic risk) and enteric bacteria (environmental risk) in intestinal homeostasis, the molecular mechanisms remain unknown. A Drosophila model with powerful genetic approaches might provide clues to the mechanisms.

As summarized here, autophagy has a critical role in anti-bacterial and anti-viral immunity. Although its role in animal survival is clear, autophagy is not a perfectly efficient mechanism for eliminating microbes, and thus some microbes evade autophagy to replicate in the host cells. The ability to avoid autophagy is not just dependent on the microbe species, but it is often observed that a portion of one kind of bacterium, such as Listeria, is trapped by autophagosomes, while others are able to evade autophagy. This might be because autophagy is primarily a host catabolic system that functions in the innate immune response using PRRs or other triggers for selectivity.

Because of the highly conserved systems of innate immunity between species, the genetic tools available for Drosophila will facilitate significant progress in understanding the role of autophagy against microbe infection. Further studies on autophagic function in innate immunity will provide new insights for clinical application.

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