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Autophagic targeting and avoidance in intracellular bacterial infections

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Eukaryotic cells use autophagy to break down and recycle components such as aggregated proteins and damaged organelles. Research in the past decade, particularly using *Salmonella enterica* serovar Typhimurium as a model pathogen, has revealed that autophagy can also target invading intracellular bacterial pathogens for degradation. However, many bacterial pathogens have evolved mechanisms that allow for evasion of the autophagic pathway, such as motility or direct and irreversible cleavage of proteins that comprise the autophagic machinery. As a complete and detailed understanding of the autophagic pathway and its derivatives continues to develop, it is likely that other mechanisms of inhibition by bacterial pathogens will be discovered.

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Introduction

Cells use a specialized form of autophagy, known as xenophagy, to direct intracellular pathogens for degradation in the lysosome. Studies of intracellular bacterial pathogens have described the degradation of some species by the autophagic pathway, and the mechanisms by which bacteria may escape, avoid, or inhibit autophagic targeting. Eukaryotic host cells appear to have evolved specific mechanisms to target bacterial pathogens for xenophagy, however, host pathways that recognize bacterial pathogens for autophagic targeting are not completely defined. Here, we review mechanisms of autophagic targeting and avoidance and discuss remaining questions surrounding how the cell senses and targets bacterial invaders to the autophagic pathway.

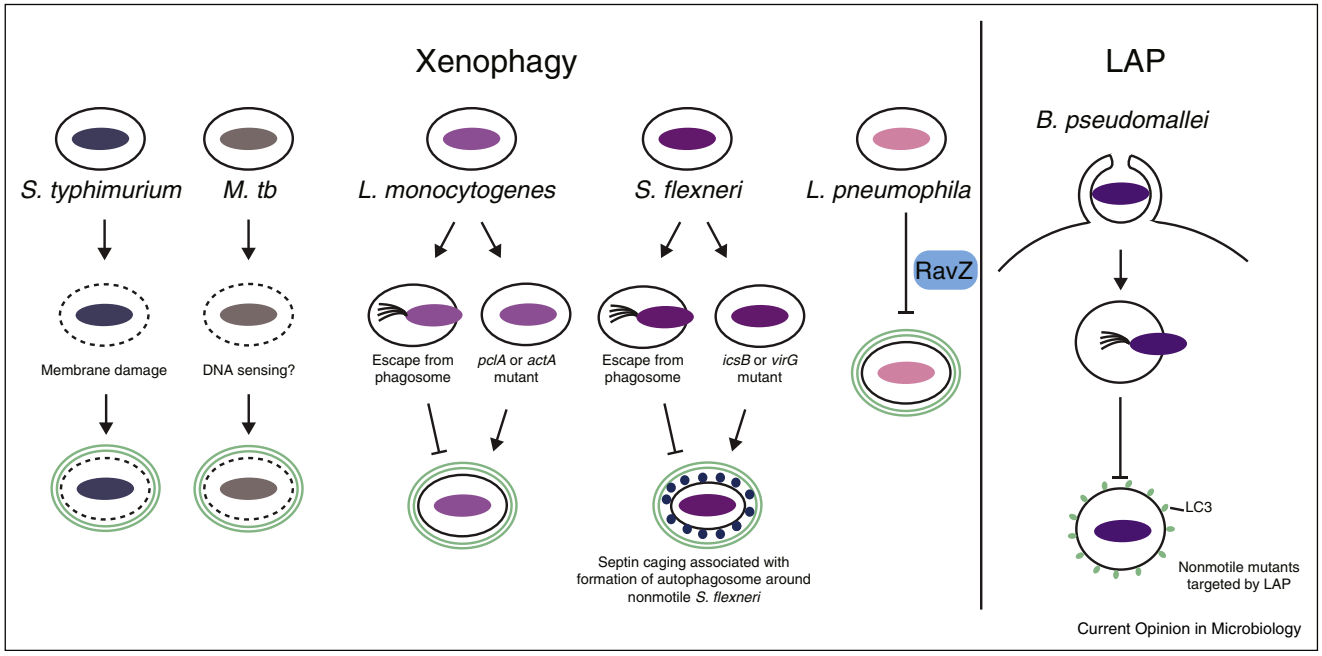
Autophagic targeting of bacterial pathogens

Eukaryotic cells recycle unneeded components, such as damaged organelles or protein aggregates, with autophagy. Although various forms of autophagy exist, here we focus on macroautophagy, which is henceforth referred to as autophagy for simplicity. Multiple signals may induce autophagy, such as starvation or inactivation of mTORC1, via signaling events upstream of autophagy initiation [1,2]. When autophagy is induced, an isolation membrane envelops cytosolic cargo, resulting in formation of a double-membrane bound organelle called an autophagosome. The membrane is derived primarily from the endoplasmic reticulum (ER) [3,4], but may also originate from ER-mitochondria contact sites and/or ER-Golgi contact sites [5,6]. In canonical models of autophagy, cargo are ubiquitinated, and then adapter proteins bind these ubiquitin residues and also Atg8 proteins on the isolation membrane [7]. The most common Atg8 protein that is monitored to assess autophagy in mammalian cell lines is called microtubule-associated protein 1A/1B-light chain 3 (LC3) [8,9]. The autophagosome then fuses with a lysosome where the cargo is degraded.

Intracellular bacterial pathogens are one type of substrate that the autophagy machinery targets for degradation (Figure 1). When autophagy specifically targets an invading pathogen, the process is called xenophagy. The most studied model is infection with *Salmonella enterica* serovar Typhimurium. Proteins involved in autophagy have been shown to influence *S. typhimurium* replication and interact with the *Salmonella*-containing vacuole (SCV). LC3, ubiquitin, and a number of adapter proteins, such as NDP52, p62/SQSTM1, TAX1BP1, and optineurin, all localize to the SCV. Silencing or deletion of genes required for autophagy results in increased bacterial replication during infection in mice, *Caenorhabditis elegans*, *Drosophila melanogaster*, and tissue culture cell lines [10–15].

Although the association of the autophagic machinery and involvement of it in reducing bacterial replication is well established, signals involved in sensing intracellular bacteria and targeting them for envelopment by autophagosomes are not fully understood. The E3 ubiquitin ligase, LRSAM1, is important for the ubiquitination involved in xenophagy of *S. typhimurium* [16]. How LRSAM1 may link to upstream cellular signaling pathways is not clear in all cases. There are likely other unidentified E3 ligases that participate in ubiquitination of *S. typhimurium*. Moreover, the exact site of ubiquitination on the bacteria itself or the vacuole membrane, has

Figure 1



Interactions of different bacterial species with autophagy. Autophagosomes form around *S. typhimurium* and *M. tb* containing phagosomes in response to either membrane damage or DNA sensing in each infection, respectively. *L. monocytogenes* and *S. flexneri* avoid xenophagy with actin tail motility. Nonmotile mutants or *pclA* mutants of *L. monocytogenes* that cannot break out of the LCV are targeted for xenophagy. Nonmotile mutants of *S. flexneri* are targeted for xenophagy through a mechanism involving septin caging. *L. pneumophila* avoids xenophagy with the T4SS effector protein RavZ, which is a protease that irreversibly cleaves lipidated Atg8 proteins. Nonmotile mutants of *B. pseudomallei* are targeted for LAP. Motile *Burkholderia* species escape the phagosome with actin tail motility.

not been clearly defined. Importantly, ubiquitin-independent mechanisms for autophagic targeting also exist. In *S. typhimurium* infected cells, a subset of bacteria disrupt vacuoles in which they reside. The host protein, Galectin-8, detects damaged membranes by binding to host glycans displayed by proteins in the lumen that are exposed upon vacuole rupture. The autophagy adapter protein NDP52 binds Galectin-8 and Atg8 proteins on an isolation membrane to direct the formation of an autophagosome around the damaged vacuole [17•]. Ubiquitin-mediated processes can amplify detection that occurs through the Galectin-8 pathway. Additionally, sensing of diacylglycerol (DAG) to target *S. typhimurium* for autophagy has been proposed as an ubiquitin-independent sensing mechanism [18]. It remains unclear if other sensing mechanisms exist.

Like *S. typhimurium*, there are other examples of pathogenic bacteria that can be targeted by autophagy during infection. Induction of autophagy with rapamycin, an inhibitor of mTOR, promotes restriction of *Mycobacterium tuberculosis* during infection in macrophages [19]. Mice containing myeloid cells deficient in *Atg5* are more susceptible to *M. tb* infection and display dampened levels of inflammation [20]. LC3, adapter proteins, and ubiquitin all localize to the *M. tb* containing vacuole [21]. Furthermore, Ubiquilin 1 (UBQN1) and the E3 ubiquitin ligase Parkin independently direct the autophagic machinery to *M. tb* [22,23]. Similarly, Group A *Streptococcus pyogenes* (GAS) is enveloped by double-membrane bound autophagosomes after escape from the pathogen-containing vacuole resulting in bacterial degradation. Infection of *Atg5*−/− cells with GAS results in increased bacterial numbers [24••]. As during infection with *S. typhimurium*, other upstream signals that may be involved in autophagic targeting are undefined for *M. tb* and *S. pyogenes*.

In addition to selective autophagy, cargo can also be subject to degradation after targeting by a non-conventional form of autophagy known as LC3-associated phagocytosis (LAP). During LAP, the pathogen does not disrupt the vacuole in which it resides and remains within a single membrane bound compartment after phagocytosis by the host cell. LC3 is conjugated to the membrane of the phagosome immediately after phagocytosis. The LC3-positive compartment then rapidly fuses with the lysosome [25••,26]. Importantly, the protein Rubicon is required for LAP but Rubicon is not required for conventional xenophagy of intracellular pathogens [26]. Non-motile mutants of *Burkholderia pseudomallei* have been shown to be targeted by LAP [27]. Importantly, LAP can be triggered when cargo interacts with Toll-Like Receptors (TLR) prior to phagocytosis, suggesting a link between the innate immune system and this