CHAPTER FOUR

The interplay between selective types of (macro)autophagy: Mitophagy and xenophagy

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Abstract

Autophagy is a physiological response, activated by a myriad of endogenous and exogenous cues, including DNA damage, perturbation of proteostasis, depletion of nutrients or oxygen and pathogen infection. Upon sensing those stimuli, cells employ multiple non-selective and selective autophagy pathways to promote fitness and survival.

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Importantly, there are a variety of selective types of autophagy. In this review we will focus on autophagy of bacteria (xenophagy) and autophagy of mitochondria (mitophagy). We provide a brief introduction to bulk autophagy, as well as xenophagy and mitophagy, highlighting their common molecular factors. We also describe the role of xenophagy and mitophagy in the detection and elimination of pathogens by the immune system and the adaptive mechanisms that some pathogens have developed through evolution to escape the host autophagic response. Finally, we summarize the recent articles (from the last five years) linking bulk autophagy with xenophagy and/or mitophagy in the context on developmental biology, cancer and metabolism.

1. Introduction

During evolution, cells have developed a plethora of mechanisms to adapt to endogenous deleterious stressors (i.e. DNA damage, proteotoxic stress) and changing environments (i.e. nutrients depletion or hypoxic conditions). Macroautophagy (thereafter referred to as autophagy) is a key adaptive mechanism to overcome these insults. A plethora of distinct autophagic procedures have been defined, including xenophagy and mitophagy, among others (Galluzzi et al., 2017). Here we report the most recent advances in the study of autophagy, with special focus in xenophagy and mitophagy and how their crosstalk regulates a wide range of cellular processes, including ageing, metabolism, and cancer. The review aims to offer a general overview of these topics, with special focus on (but not limited to) the literature from the last 5 years.

1.1 Autophagy

Autophagy is a physiological cellular process for the intracellular degradation of aberrant proteins, damaged organelles or intracellular pathogens. In addition to protein aggregates, injured organelles and infectious agents, autophagy can be activated by a plethora of stimuli, including nutrient starvation, growth factor deprivation, hypoxia, reactive oxygen species and DNA damage. Thus, autophagy entails an adaptive mechanism to alleviate cellular stresses, obtain energy, and, ultimately, promote cell survival. Canonical autophagy is mediated by double membrane vesicles called autophagosomes that sequester cellular components and deliver them to the lysosome for degradation. Autophagy plays a key role in many biological processes, ranging from development to tumorigenesis. Originally, autophagy was perceived as a bulk non-selective process, through which cytoplasmic material is indiscriminately recycled to provide energy and

building blocks. Nevertheless, it is now appreciated that autophagy operates in a highly selective manner, and a variety of selective autophagy pathways have been defined. According to the cargo of selective autophagy, these include mitophagy, nucleophagy, pexophagy, reticulophagy/ER-phagy, lipophagy, aggrephagy, ferritinophagy and more. Apart from degrading endogenous material, autophagy (originating from the Greek words "auto" meaning "self" and "phagy" meaning eating) may also target exogenous material, including bacteria, viruses and fungi, in a process termed xenophagy (originating from "xeno" meaning "foreign") (Deretic and Kroemer, 2022; Gatica et al., 2018; Yu et al., 2018).

1.2 Mitophagy

Mitophagy is a type of selective autophagy that clears dysfunctional, old or excessive mitochondria by targeting them for degradation in autophagosomes. Hence, mitophagy promotes cellular homeostasis by maintaining the integrity of the mitochondrial pool and/or adapting the mitochondrial content to certain stresses, similar to those triggering autophagy (Markaki and Tavernarakis, 2020). As expected, mitophagy molecular cascades overlap with those involved in other types of selective autophagy, as well as in bulk autophagy. Autophagy and mitophagy initiation seem to be triggered, at least partially, by the same molecular components (reviewed in (Zachari and Ktistakis, 2020)). In autophagy, the mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) transmit signals of stress via its downstream effector the unc-51 like autophagy activating kinase 1 (ULK1) complex. Thus, upon certain stresses, ULK1 complex promotes autophagy initiation. Similarly, the ULK1 complex has been described to coordinately colocalize with autophagy-related protein 9 (ATG9) vesicles along the endoplasmic reticulum (ER). ATG9 vesicles are another source of lipid membrane that is included in new autophagosomes and mitophagosome (i.e. autophagosomes engulfing mitochondria) initiation (Lou et al., 2020). Therefore, both ULK1 complex and ATG9 vesicles are key inducers for mitophagosome initiation (Yamano et al., 2018). The associated ATG machinery also overlaps with that used in autophagy and, in a similarly way to autophagy, once the machinery is properly localized, the phagophore engulfing the mitochondria elongates and matures via the addition of lipids until it is ready to be closed by the endosomal sorting complex (reviewed in Killackey et al., 2020). Mitophagy is promoted via ubiquitination of outer mitochondrial membrane proteins or through the

function of selective mitophagy receptors, such as BCL2 and adenovirus E1B 19 kDa-interacting protein 3 (BNIP3) and Nip3-like protein X (NIX)/BNIP3-like protein (BNIP3L), as recently reviewed elsewhere (Onishi et al., 2021).

1.3 Xenophagy

Xenophagy is a type of selective autophagy targeting invading pathogens, acting via ubiquitin-dependent and independent pathways. During host cell invasion, bacteria are initially encapsulated into single membrane vacuolar compartments. However, invading pathogens may breach the vacuole and gain access to the nutrient rich cytosol. As a result, pathogens themselves, as well as the internal components of the vacuolar membrane, get exposed to the cytosol, where they are recognized as intruders by diverse host systems and initiate multiple host defense pathways (Reggio et al., 2020). Glycans are key constituents of the vacuolar membrane that are recognized by galectins, which are host proteins that act as bacterial sensors. Several galectins, including galectin 3, galectin 8 and galectin 9 are involved in xenophagic responses in multiple ways, for instance by recruiting autophagy receptors, such as nuclear dot protein 52 (NDP52)/calcium binding and coiled-coil domain 2 (CALCOCO2) and tripartite motif-containing (TRIM) proteins (Chauhan et al., 2016; Johannes et al., 2018; Thurston et al., 2012). These receptors directly bind to members of the microtubule associated protein 1 light chain 3 (MAP1LC3) and GABA type A receptor-associated protein (GABARAP) families and deliver the cargo to autophagosomes. Furthermore, additional components of the vacuolar compartment can recruit core autophagy proteins to induce xenophagy. For example, the V-ATPase of the vacuolar membrane physically interacts with autophagy related 16 like 1 (ATG16L1) through its WD40 domain, to promote bacterial autophagy during *Salmonella* infection (Xu et al., 2019).

Recent evidence supports that multiple autophagy receptors may be recruited in a sequential fashion on bacteria-containing vacuoles or intracellular pathogens. Toll interacting protein (TOLLIP), an autophagy receptor previously shown to be involved in the clearance of protein aggregates, was recently identified as a critical regulator of bacterial autophagy during group A *Streptococcus* (GAS) infection. Specifically, TOLLIP accumulates on to GAS-containing vacuoles prior to their rupture, where it promotes the recruitment of additional autophagy receptors, such as neighbor of

BRCA1 gene 1 (NBR1), tax1 binding protein 1 (TAX1BP1) and NDP52, as well as several galectins, to promote xenophagy (Lin et al., 2020).

Apart from delivering the cargo into autophagosomes, novel studies have established unexpected roles for cargo receptors in xenophagy. For instance, during *Salmonella* infection the autophagy receptor NDP52 orchestrates the *de novo* biogenesis of phagophores in the bacterial vicinity and juxtapositions phagophores and cargos. Mechanistically, NDP52 forms a tripartite complex with 200 kDa FAK family kinase-interacting protein (FIP200) and similar to NAP1 TBK1 adaptor (SINTBAD) – NAK associated protein 1 (NAP1) via its N-terminal SKIP carboxyl homology (SKICH) domain, independently of its LC3-interacting region (LIR). These in turn recruit ULK1 and tank-binding kinase 1 (TBK1) respectively to initiate bacterial autophagy (Ravenhill et al., 2019).

2. Xenophagy and mitophagy act through common molecular mechanisms

Remarkably, xenophagy and mitophagy operate, at least partially, via shared molecular mechanisms. Apart from using the core autophagy machinery, these two selective autophagy pathways employ common molecules, including autophagy receptors and E3 ubiquitin ligases, to achieve cargo selectivity (Goodall et al., 2022).

2.1 Autophagy receptors

Autophagy receptors are key mediators of autophagy selectivity. These proteins are characterized by their ability to bind cargo and facilitate the recruitment of the autophagic machinery. Cargo binding may occur directly or through the recognition of attached polyubiquitin chains, via the ubiquitin binding domain (UBD) of receptors. On the other hand, the LC3-interacting region (LIR) and/or FIP200-interacting region (FIR) motifs of the receptors orchestrate the *in situ* recruitment of expanding phagophores and/or the *de novo* formation of autophagosomes in the vicinity of cargo (Kirkin and Rogov, 2019). The list of shared autophagy receptors between xenophagy and mitophagy pathways includes NDP52, optineurin (OPTN) and TAX1BP1, among others.

The role of NDP52 in xenophagy was originally identified by Thurston et al., as they described its function during *Salmonella enterica serovar Typhimurium* (*S. typhimurium*) infection. NDP52 recognizes ubiquitin-coated cytosolic bacteria and recruits a molecular complex comprised of

TBK1, NAP1 and SINTBAD. This complex functions to deliver *S. typhimurium* into autophagosomes, thereby restricting intracellular bacterial proliferation and serving innate immunity (Thurston et al., 2009). NDP52 also binds to galectin 8, a constituent of the Salmonella containing vacuoles, targeting the bacteria-related structures for autophagic degradation (Thurston et al., 2012). NDP52 is involved in the autophagic degradation of other cytosolic bacteria as well, such as *Shigella flexneri*, *Listeria monocytogenes* and *Mycobacterium tuberculosis* (Manzanillo et al., 2013; Mostowy et al., 2011). Apart from mediating delivery of bacterial cargo to autophagosomes, NDP52 has additional roles in bacterial autophagy. Specifically, it has been shown to promote autophagosomal maturation, via an mechanism entailing interaction with myosin VI and LC3C, through the myosin VI binding domain and the non-canonical LIR motif (CLIR) respectively (Verlhac et al., 2015; Von Muhlinen et al., 2012). Recently, a novel role of NDP52 in the xenophagy pathway was described, as it was shown that NDP52 recruits the upstream autophagy machinery, by directly interacting with FIP200 and SINTBAD via the N-terminal SKITCH domain, during *S. typhimurium* infection (Ravenhill et al., 2019).

OPTN is another autophagy receptor involved in xenophagy. OPTN recognizes ubiquitin-coated cytosolic *S. typhimurium*, via its ubiquitin binding in ABIN and NEMO (UBAN) domain. Importantly, OPTN gets phosphorylated within the LIR domain on Ser177 by TBK1, a post-translational modification that increases its affinity to LC3 by several folds and promotes autophagic degradation of cytosolic bacteria (Wild et al., 2011). The TBK1-OPTN axis is not specific for the clearance of *Salmonella* bacteria, but also mediates the autophagic targeting of *Listeria monocytogenes* (Puri et al., 2017).

TAX1BP1, a paralogue of NDP52, was identified as a xenophagy receptor by Tumbarello et al. Specifically, TAX1BP1 is recruited to ubiquitinated *Salmonella*, where it functions in a partially overlapping and redundant manner with NDP52 (Tumbarello et al., 2015). TAX1BP1 also interacts with myosin VI via the two C-terminal zinc finger domains. Interestingly, TAX1BP1 binds to several members of the mammalian ATG8 family proteins, including LC3A, LC3C, GABARAPL1 and GABARAPL2. The implication of TAX1BP1 in multiple selective autophagy pathways, including xenophagy and mitophagy, has been recently reviewed elsewhere (White et al., 2022).

Apart from regulating autophagic degradation of bacterial pathogens, NDP52, OPTN and TAX1BP1 can target damaged mitochondria for

degradation during mitophagy. Specifically, all three receptors are recruited to depolarized mitochondria in a PINK1-dependent manner and facilitate their degradation (Heo et al., 2015; Lazarou et al., 2015; Wong and Holzbaur, 2014). In contrast to their function during xenophagy, NDP52 and OPTN act redundantly in mitophagy (Lazarou et al., 2015). In addition to recognizing ubiquitinated proteins on the outer mitochondrial membrane, recent studies support that NDP52 and OPTN can be recruited during later steps of mitophagy independently of their ubiquitin binding domains, via LIR-mediated interactions, to amplify mitophagy through a positive feedback loop (Padman et al., 2019). Besides, NDP52 can also invade depolarized mitochondria and interact with mitochondrial RNA poly(A) polymerase (MTPAP) via the SKICH domain. The internal NDP52-MTPAP complex acts as an autophagy receptor, similarly to the inner mitochondrial receptor prohibitin 2 (PHB2) (Furuya et al., 2018; Wei et al., 2017).

In congruence with its implication in xenophagy, TBK-1 is a critical regulator of mitophagy, as it phosphorylates all three above-mentioned receptors in order to modify their properties and create signal amplification loops. TBK1 phosphorylates OPTN on multiple sites, including Ser473, Ser177 and Ser513, to stabilize it on depolarized mitochondria, increase its affinity to LC3 and expand its binding capacity to diverse polyubiquitin chains (Heo et al., 2015; Lazarou et al., 2015; Moore and Holzbaur, 2016; Richter et al., 2016). Moreover, recent evidence supports that TBK1-mediated phosphorylation of NDP52 promotes its interaction with FIP200 and focal biogenesis of autophagosomes around depolarized mitochondria, thereby providing a mechanism for ULK1 recruitment and activation in a nutrient-rich environment during selective autophagy (Vargas et al., 2019).

2.2 E3 ligases

Ubiquitination, a seminal process in essentially all selective autophagy pathways, serves as an "eat-me" signal to mediate cargo recognition by the autophagic machinery (Gatica et al., 2018). Substrate mono- and polyubiquitination occurs by the coordinated function of E1 ubiquitin activating enzymes, E2 ubiquitin conjugating enzymes and E3 ligases. Ubiquitin ligases comprise a large heterogeneous family of approximately 600 enzymes, displaying significant functional and structural specificity (Yau and Rape, 2016). Numerous E3 ligases have well-established roles in selective autophagy, including xenophagy and mitophagy (summarized in Fig. 1 and recently reviewed in (Jetto et al., 2022, Tripathi-Giesgen et al., 2021))

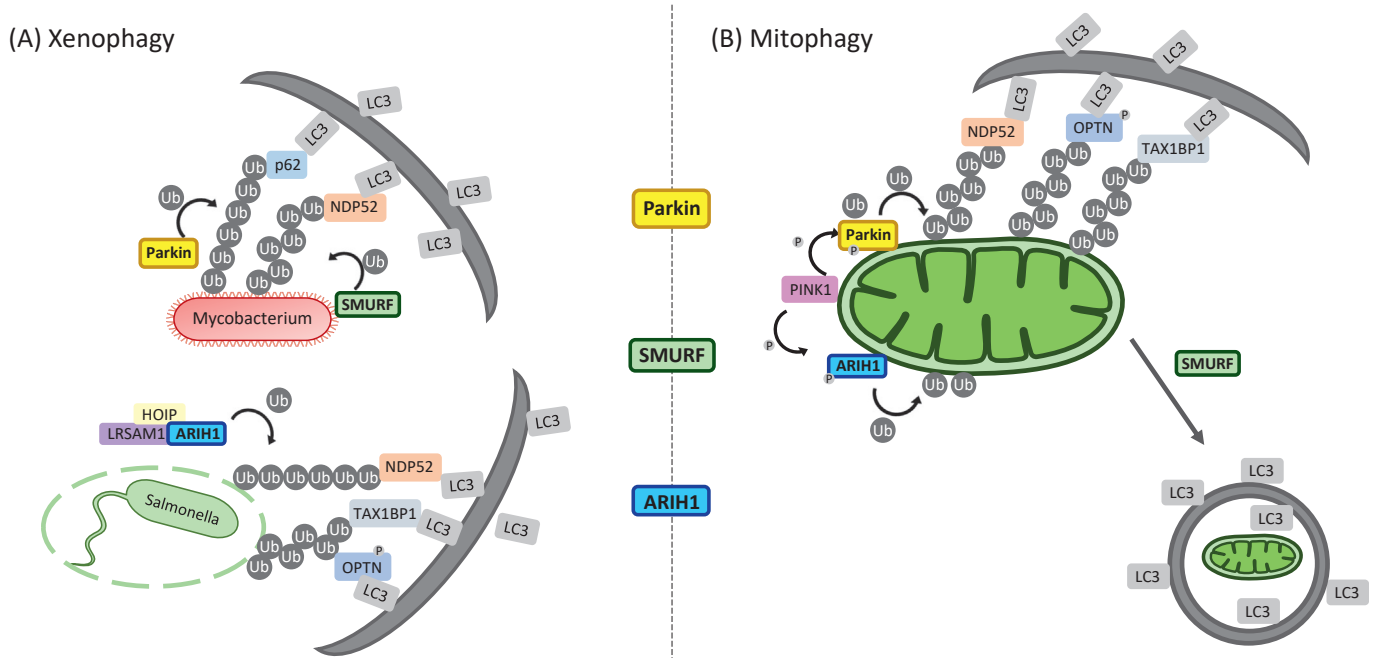

Fig. 1 Shared E3 ligases between xenophagy and mitophagy. Parkin, SMURF and ARIH1 orchestrate autophagic clearance of invading bacteria and depolarized mitochondria. (A) During xenophagy Parkin and SMURF generate polyubiquitin chains on cytosolic *Mycobacterium tuberculosis*. ARIH1, in complex with two additional E3 ligases, LRSAM1 and HOIP, polyubiquitinates cytosolic *Salmonella enterica serovar Typhimurium*. The newly-formed ubiquitin coat serves as a platform for the recruitment of autophagy receptors, including p62, NDP52, TAX1BP1 and OPTN to promote autophagic targeting and degradation. (B) Upon mitochondrial depolarization, the PINK1 kinase is stabilized in the outer mitochondrial membrane, where it recruits and phosphorylates the E3 ligases Parkin and ARIH1. These in turn ubiquitinate several outer mitochondrial membrane proteins, which recruit autophagy adaptors to induce mitophagy. SMURF facilitates mitochondrial delivery into autophagosomes, independently of its ubiquitin ligase activity.

Parkin is probably the most-studied E3 ubiquitin ligase in selective autophagy, mainly due to its key involvement in the PINK1-parkin mitophagy pathway (Narendra et al., 2008). Parkin is a promiscuous enzyme, targeting numerous molecules, including itself. During mitophagy, parkin is recruited on the outer mitochondrial membrane, through direct phosphorylation by PINK1 on Ser65 in the ubiquitin-like (Ubl) domain, as well as through interaction with phosphorylated ubiquitin. These two steps increase parkin's ligase activity, which once fully activated ubiquitinates a wide range of outer mitochondrial membrane proteins (Sarraf et al., 2013). In addition to mitophagy, parkin is also involved in xenophagy, specifically during Mycobacterium tuberculosis infection, where it polyubiquitinates cytosolic bacteria and facilitates the recruitment of the autophagy receptors p62 and NDP52 (Manzanillo et al., 2013).

Ariadne RBR E3 Ub protein ligase 1 (ARIH1), also known as HHARI, is an E3 ligase recently identified as a critical regulator of mitophagy in cancer cells, which inherently have downregulated parkin expression. ARIH1-mediated mitophagy also depends on PINK1, but is independent of NDP52 and OPTN (Villa et al., 2017). Up to date, the mitochondrial substrates of ARIH1, as well as the type of polyubiquitin chains assembled by this E3 ligase during mitophagy remain uncharacterized. Regarding xenophagy, ARIH1 was shown to participate in cytosolic *S. typhimurium* ubiquitination, acting in coordination with two additional E3 ligases, namely leucine rich repeat and sterile alpha motif containing 1 (LRSAM1) and HOIL-1-interacting protein (HOIP). These three ubiquitin ligases form a cooperative network that decorates invading *Salmonella* with K48-, K63- and M1- polyubiquitin chains (Polajnar et al., 2017). The role of M1-linked linear polyubiquitin chains in xenophagy has been recently reported, whereas to date there is no evidence for linear ubiquitination in mitophagy (Noad et al., 2017; Van Wijk et al., 2017).

SMAD-specific E3 ubiquitin-ligase protein 1 (SMURF1) is another shared molecule between xenophagy and mitophagy. Upon *Mycobacterium tuberculosis* and *Listeria monocytogenes* infection, SMURF1 generates K48-linked polyubiquitin chains through a mechanism requiring both its ubiquitin-ligase and C2 phospholipid-binding domains (Franco et al., 2017). An earlier study by the same group has shown that SMURF1 also participates in mitophagy, yet in this pathway SMURF1 functions independently of its E3 ubiquitin ligase activity. Instead, it was reported that SMURF1 is involved in the delivery of mitochondria to autophagosomes, in an uncharacterized manner depending on the C2-membrane targeting domain (Orvedahl et al., 2011).

Importantly, autophagy, including xenophagy and mitophagy, are of utmost importance in controlling immune responses, developmental processes, metabolism and carcinogenesis.

3. Immunity and disease

3.1 Xenophagy and mitophagy cooperate in pathogen elimination and generation of antigens for adaptive immunity activation

The immune system consists of two branches: the innate immune response, which constitutes a rapid first line of defense, and the antigen-mediated acquired or adaptive immunity. The main characteristics, cell types and molecular pathways mediating each immune response have been extensively reviewed elsewhere (Chaplin, 2010; Marshall et al., 2018; Tan et al., 2015). Both xenophagy and mitophagy are involved in the immune detection and elimination of pathogens, specifically in the innate immune response (Gkikas et al., 2018; Levine et al., 2011).

Recent publications point to a diversity of xenophagy- and mitophagy-related mechanisms to detect pathogens. In this sense, Radomski et al. proposed a novel mito-xenophagic pathway connecting innate and adaptive immunity. They studied the bacterial pathogens Chlamydiae, which are Gram-negative obligate intracellular bacteria pathogens. They predominantly infect human and animal epithelial mucosae, where they grow in vacuolar inclusions. The mito-xenophagic pathway suggested by Radomski et al. involves autophagy of bacterial components, as well as mitochondrial modulation of the development and integrity of the energy-dependent parasitic inclusions. This mechanism relies on cytoprotective heat shock protein 25/27 (HSP25/27), E3 ubiquitin ligase parkin, and histone deacetylase 6 (HDAC6), to potentiate the generation of chlamydial antigens for its presentation to dendritic cells via major histocompatibility complex I (MHC I) (Radomski et al., 2017).

It is important to highlight that hijacking host autophagy is a mechanism related to immune escape. The term immune escape refers to the ability of pathogens to hamper their detection and elimination by the immune system by different means. As a result of host-pathogen co-evolution, pathogens have developed a plethora of mechanisms to promote immune escape and, in many cases, to increase transmission and virulence (Finlay and McFadden, 2006; Rosbjerg et al., 2017; Sasaki et al., 2022).

Remarkably, pathogens have evolved to avoid host autophagic degradation, thereby promoting infection. Ogawa et al. identified the mechanism by which *Shigella flexneri*, a pathogenic agent causing diarrhea in humans, escapes host autophagic response. This Gram-negative invasive bacterium injects IcsB effector protein into host cells through a macromolecular protein nano-syringe called the type III secretion system, thereby escaping host autophagy. Mutant bacteria lacking IcsB are defective in multiplication and spreading within host cells, due to their efficient elimination by autophagy. Mechanistically, IcsB suppresses autophagy through binding to VirG, another *Shigella flexneri* protein required for intracellular actin-based motility. Hence, IcsB binding to VirG competitively inhibits binding of VirG to the autophagy host protein ATG5, leading to host autophagic response scape (Ogawa et al., 2005) (Fig. 2).

Moreover, a study from Shizukuishi et al. provided another example of a pathogen targeting host autophagy. *Streptococcus pneumonia* is a Gram-positive bacterium causing Pneumococcal infections. Shizukuishi et al. discovered that Pneumococcal cell surface-exposed choline-binding protein (CBP) C (CbpC) from *S. pneumoniae* strain TIGR4 activates autophagy via interaction with the autophagy host protein ATG14 (Shizukuishi et al., 2020).

Importantly, there is compelling literature on the mechanisms by which pathogens escape from autophagy and this topic has been reviewed elsewhere (Huang and Brumell, 2014; Riebisch et al., 2021; Siqueira et al., 2018).

3.2 Pathogens promote mitophagy to limit xenophagy

As previously mentioned, mitochondria play a key role in regulating various cellular physiological activities, as well as immune responses (reviewed in Gkikas et al., 2018, Tiku et al., 2020). Specifically, during infections, mitochondria produce reactive oxygen species (ROS), which can kill the intruding bacteria or halt their proliferation. Therefore, pathogens have evolved to utilize sophisticated strategies to perturb mitochondrial function, in order to evade host immune responses.

Several bacterial pathogens can alter mitochondrial dynamics, through diverse molecular mechanisms (Fig. 3). For instance, the intracellular Gram-positive bacterium *Listeria monocytogenes* promotes mitochondrial fragmentation independently of dynamin-related protein 1 (Drp-1) (Stavru et al., 2013). Oppositely, *Helicobacteri pylori* and *Shigella flexneri* engage Drp-1-dependent mechanisms to promote mitochondrial fission

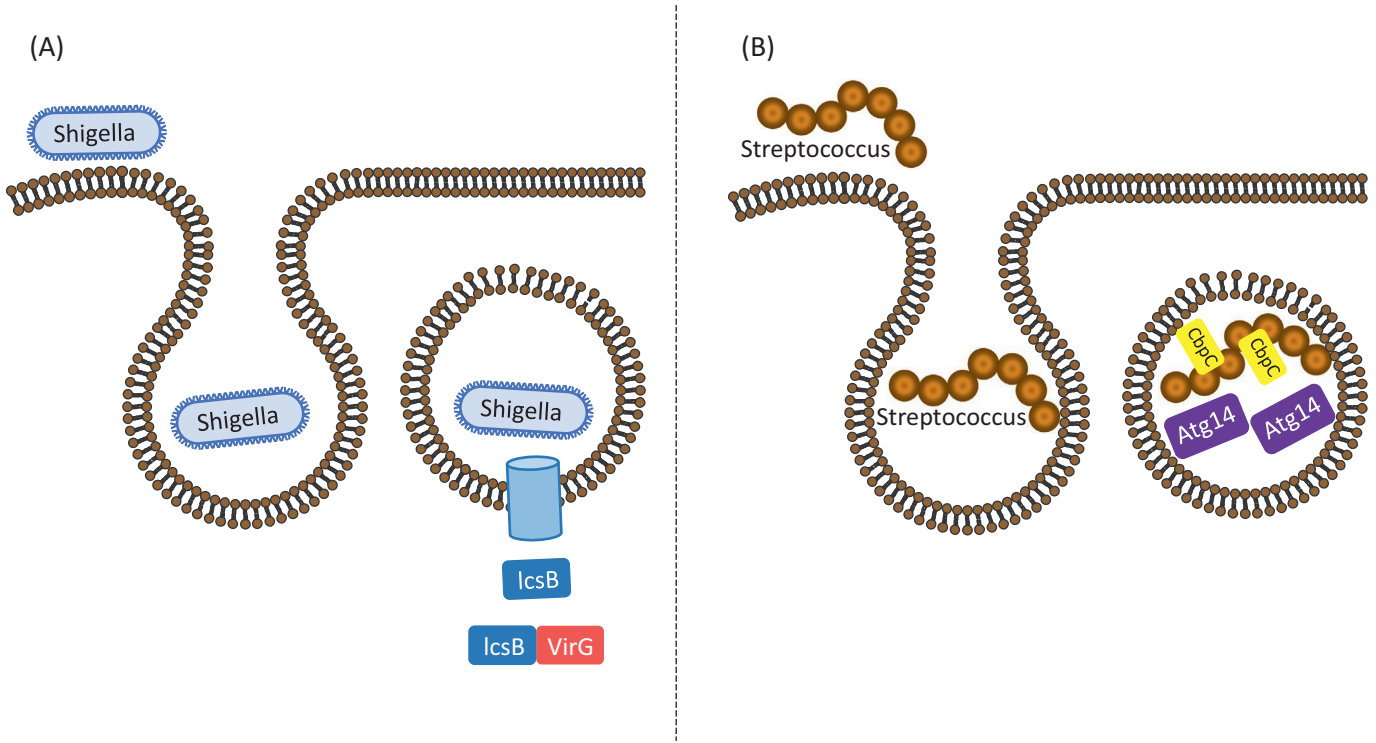

Fig. 2 Autophagy-related mechanisms of immune escape. (A) *Shigella flexneri* escapes host autophagic response by injecting IcsB effector protein into host cells, which interacts with VirG, whereas (B) *Streptococcus pneumonia* activates host autophagy via interaction of choline-binding protein (CBP) C (CbpC) with the autophagy host protein ATG14.

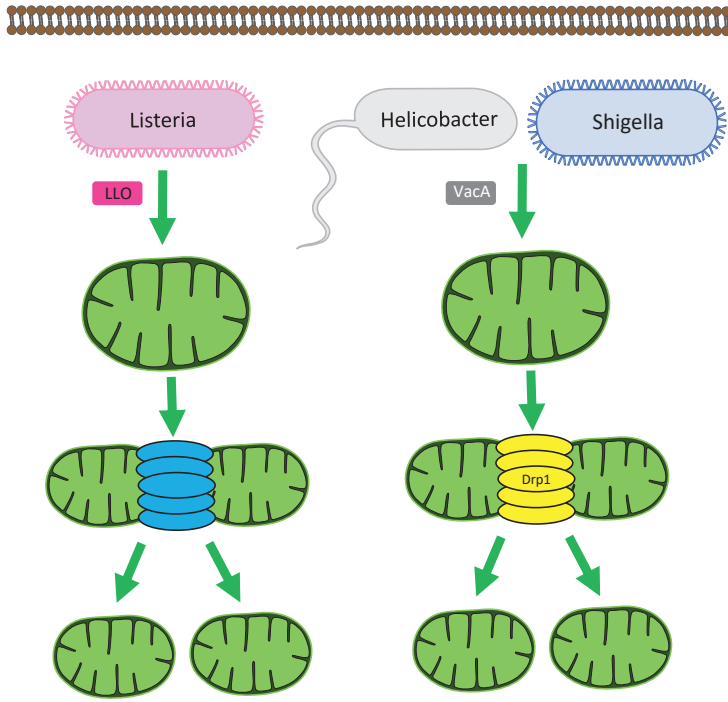

Fig. 3 Pathogens promote mitochondrial fission. *Listeria monocytogenes* induces secreted pore-forming toxin listeriolysin O (LLO)-mediated mitochondrial fragmentation. Mitochondrial fragmentation occurs even in the absence of functional dynamin-like protein 1 (Drp1), a component of the canonical mitochondrial fission machinery. On the contrary, *Helicobacteri pylori* (via vacuolating cytotoxin A [VacA]) and *Shigella flexneri* activate Drp-1-dependent mechanisms to cause mitochondrial fission.

(Jain et al., 2011; Lum and Morona, 2014). In all cases, bacteria enhance mitochondrial fragmentation to impede mitochondrial function and compromise host defense.

Apart from perturbing mitochondrial function, mitochondrial fragmentation has been shown to facilitate mitophagy in numerous experimental settings, as it allows the generation of smaller fragments that are amenable to sequestration into autophagosomes (Palikaras et al., 2018). Thus, mitochondrial fission could also be triggered by invading pathogens to induce autophagic clearance of mitochondria and, thereby, reduce their abundance. Indeed, several strategies are employed by bacteria to promote host mitophagy. For instance, *Listeria monocytogenes* induces mitophagy in macrophages, a process that facilitates its intracellular survival and proliferation.

Mechanistically, *L. monocytogenes* promotes the oligomerization and activation of the Nod-like receptor X1 (NLRX1), which is a novel mitophagy receptor necessary for pathogen-induced mitophagy. Notably, other well-established mitophagy receptors, such as BNIP3, BNIP3L and FUNDC1, as well as the PINK1/parkin pathway, are not implicated in *L. monocytogenes*-induced mitophagy (Zhang et al., 2019).

Mitophagy and xenophagy share numerous molecular components, including autophagy receptors/adaptor proteins. Therefore, it is tempting to speculate that during pathogen invasion, induction of mitophagy could restrict the availability of shared resources and thus limit xenophagy. This holds true for *Mycobacterium bovis,* which triggers PINK1/parkin-dependent mitophagy to suppress xenophagy, by competitive utilizing p-TBK1. Inhibition of mitophagy increases the colocalization of p-TBK1 with *M. bovis* and suppresses bacterial growth, while additional activation of mitophagy using CCCP decreases their colocalization and reduces virulence. Therefore, *Mycobacterium bovis*-induced mitophagy inhibits host xenophagy, resulting in enhanced intracellular survival of the pathogen (Song et al., 2021).

These data highlight the existence of a wide range of molecular mechanisms leading to autophagic degradation of mitochondria during pathogen infection. Similar strategies are employed by numerous viruses, which activate mitophagy to circumvent host immune responses (reviewed in Zhang et al., 2018).

3.3 Autophagy and mitophagy play a key role in development

Autophagy and mitophagy have been found to play a role in a wide range of developmental processes (this topic has been recently reviewed somewhere else: Allen and Baehrecke, 2020, Hu et al., 2019, Perrotta et al., 2020). These two types of recycling processes have been found to interact in many different biological contexts, such the vertebrate eye or erythroids (recent publications regarding this topic are described below and summarized in Table 1). Indeed, the prevalence of basal mitophagy and its relationship to general autophagy have been recently studied in the visual system by McWilliams et al. They concluded that autophagy is developmentally regulated in a variety of ocular tissues and mitophagy is particularly active in the adult retina, specifically in the photoreceptor neurons of the outer nuclear layer (McWilliams et al., 2019).

Yang et al. focused on the molecular mechanisms governing terminal erythroid differentiation. They found that autophagy genes (*Atg3*, *Atg5*, *Atg7* and *Atg10*) are highly expressed during the early stage of terminal erythroid differentiation and they become downregulated along erythroblast

Table 1 Recent studies on the role of autophagy and mitophagy in developmental biology.

Topic	Model	Main findings	Reference
The visual system	Mito-QC and mCherry-GFP-LC3 mice on a C57BL6/J background	Autophagy is developmentally regulated in the vertebrate eye	McWilliams et al. (2019)
		Mitophagy is active in the adult retina, specifically in the photoreceptor neurons of the outer nuclear layer	
Erythroid differentiation	*Atg7*$^{fl/fl}$-*Mx1Cre* mice on a C57/BL6 background and mouse MEDEP-BRC5 cell line	Expression of autophagy mediators negatively correlates with erythroblast maturation, whereas mitophagy factors display the opposite expression pattern	Yang et al. (2019)
		SPHK1 controls terminal erythroid differentiation by regulating mitochondrial content though the activation of BNIP3L/NIX and PINK1 expression	

maturation. On the contrary, mitophagy factors (*Pink1*, *Park2*, *Bnip3l/Nix* and *p62/Sqstm1*) were displayed the opposite expression pattern, which positively correlated with erythroblast maturation. The authors proposed a mechanism by which Sphingosine kinase 1 (SPHK1) controls terminal erythroid differentiation by regulating mitochondrial content though the activation of BNIP3L/NIX and PINK1 expression ((Yang et al., 2019).

3.4 Autophagy and mitophagy affect carcinogenesis

Alterations in autophagy and/or basal mitochondrial turnover in response to oncogenic stresses can be beneficial or detrimental for tumor development and progression, and response to anti-cancer therapy (Guan et al., 2021; Yun and Lee, 2018) (recent publications regarding this topic are described below and summarized in Table 2). In this line, Santarelli et al. showed that xenophagy and mitophagy are targeted by the cancer-causing Kaposi's sarcoma-associated herpes (KSHV). The virus drives Kaposi's sarcoma by infecting endothelial cells of immune-compromised patients. Mechanistically, KSHV activates the molecular pathway PI3K/AKT/mTOR and its downstream targets eukaryotic translation initiation factor 4E binding protein (EIF4EBP1) and ULK1. Furthermore, it attenuates

Table 2 Recent studies on the role of autophagy and mitophagy in cancer.

Topic	Model	Main findings	Reference
Kaposi's sarcoma	Primary human umbilical vein endothelial (HUVEC) cells	Kaposi's sarcoma-associated herpes (KSHV) induces mechanistic target of rapamycin kinase (mTOR) and its downstream targets eukaryotic translation initiation factor 4E binding protein (EIF4EBP1) and unc-51-like autophagy activating kinase 1 (ULK1) to attenuate autophagy and mitophagy and promote carcinogenesis	Santarelli et al. (2020)
Cancer stem cells	P19 embryonic carcinoma stem cells	Activation of autophagy correlates with pluripotency, thus providing quality control mechanism that maintain (cancer) stemness	Magalhães-Novais et al. (2020)
		Mitophagy may contribute to the maintenance of stemness	
Adaptation to hypoxia	Human neuroblastoma (SK-N-BE(2), SH-SY5Y, TET21N), lung large cell carcinoma (U1810) and lung adenocarcinoma (A549) cells	The mitophagy receptors BNIP3 and BNIP3L facilitate elimination of proapoptotic mitochondria via mitophagy during hypoxia	Abdrakhmanov et al. (2021)
Cancer survival	Human BT549 (female) cells, and NCIH292 (female) cells, and mouse embryonic fibroblasts (MEFs)	Cancer cell lines adapt to the loss of autophagy genes by upregulating mitochondrial fusion and enhancing the formation of SNX9-mediated and LC3-independent mitochondrial-derived vesicles (MDVs)	Towers et al. (2021)
Genomic self-DNA	Human undifferentiated colon adenocarcinoma HT29 cell line	Incubation with fragmented-DNA upregulates autophagy and lipophagy, and improves tumor cell survival	Sipos et al. (2019)
		Incubation with hypermethylated-DNA decreases cancer cell survival through stimulation of apoptosis and mitophagy	

Table 2 Recent studies on the role of autophagy and mitophagy in cancer.—cont'd

Topic	Model	Main findings	Reference
Anti-cancer therapy	Human MZ-54, U87MG and U343 glioma cell lines	AT 101 ([−]-gossypol) triggers autophagic cell death, heme oxygenase 1 (HMOX1) upregulation, and mitochondrial dysfunction	Meyer et al. (2018b)
Anti-cancer therapy	Human A2780 ovarian cancer cell line	Epoxycytochalasin H enhances mitophagy and autophagy and subsequent mitochondrial- and ER stress-dependent apoptosis, which reduces cell proliferation	Wang et al. (2020)

autophagy and mitophagy. Activation of the mTOR pathway and the concomitant reduction in autophagy and mitophagy leads to the activation of key cellular processes; endothelial to mesenchymal transition, endoplasmic reticulum stress/unfolded protein response (ER-UPR), and secretion of the proangiogenic and proinflammatory chemokine C-C motif chemokine ligand 2 (CCL2) (Santarelli et al., 2020).

Other studies have identified a crosstalk between mitophagy and non-selective (macro)autophagy in stemness and cancer. For instance, activation of autophagy correlated with pluripotency and it raised the threshold for cell death activation in studies performed in P19 embryonic carcinoma stem cells. Thus, activation of autophagy has been defined as a quality control mechanism that maintain stemness. Although the same study suggested a contribution of mitophagy to maintenance of stemness, it needs to be further validated (Magalhães-Novais et al., 2020).

Remarkably, in the context of cancer, induction of mitophagy serves as an adaptation to deplete the cellular mitochondrial mass in response to specific stresses, including hypoxia (Chourasia et al., 2015). According to this concept, Abdrakhmanov et al. reported accumulation of the mitophagy receptors BNIP3 and BNIP3L in human neuroblastoma and lung carcinoma cell lines during hypoxia. These two mitophagy receptors seem to act in a partially redundant manner to facilitate elimination of proapoptotic mitochondria via mitophagy. Despite the presence of lipidated form of LC3 (LC3-II), canonical autophagy was not activated under hypoxic conditions in this experimental set up. Instead, receptor-mediated mitophagy seems to be a key mechanism to avoid cancer cell death upon hypoxia (Abdrakhmanov et al., 2021).

Moreover, Towers et al. inactivated the core autophagy genes (either ATG7 or RB1CC1/FIP200) in autophagy-dependent breast and lung cancer cell lines. Those clones that adapted to the loss of autophagy genes (i.e. maintained similar growth rates) upregulated mitochondrial fusion and enhanced the formation of SNX9-mediated and LC3-independent mitochondrial-derived vesicles (MDVs). These data suggest that acquired dependency on mitochondrial fusion is an adaptive mechanism to preserve mitochondrial function, whereas the increment in MDVs promotes mitochondrial degradation and homeostasis when mitophagy is affected. All these molecular adaptations appear in response to defects in autophagy in order to enable cancer cell survival (Towers et al., 2021).

Additionally, data from *in vitro* studies revealed that treatment of a colorectal adenocarcinoma cell line with different types of modified genomic self-DNA disturbs autophagy and/or mitophagy. Incubation with fragmented-DNA upregulated (macro)autophagy and lipophagy, and improved tumor cell survival. On the contrary, incubation with hypermethylated-DNA decreased cancer cell survival through stimulation of apoptosis and mitophagy. Nevertheless, no insights about the biological meaning of these changes in autophagy and mitophagy were provided by the authors (Sipos et al., 2019).

With respect to anti-cancer therapies, pharmacological modifiers of autophagy or mitophagy have been considered as a treatment for cancer. In addition, research using these drugs have shed light into the mechanisms by which these two processes regulate carcinogenesis. In this line, Meyer et al. treated glioma cells with the AT 101 ([−]-gossypol), a natural compound extracted/isolated from cotton seeds. AT 101 triggered autophagic cell death, heme oxygenase 1 (HMOX1) upregulation, and mitochondrial dysfunction (characterized by mitochondrial membrane depolarization and engulfment of mitochondria within autophagosomes with the subsequent reduction of mitochondrial mass and BAX- and BAK1-independent mitochondrial proteins). Remarkably, pharmacological inactivation of autophagy attenuated AT 101-promoted decrease in mitochondrial mass. Moreover, inhibitor of HMOX1 and the mitophagy receptors BNIP3 and BNIP3L counteracted AT 101-induced mitophagy. These results suggest a crosstalk between mitophagy and autophagy resulting in glioma cells death (Meyer et al., 2018b). Similarly, epoxycytochalasin H, a natural compound present in the flowering plant *Polygonatum sibiricum*, was tested in an ovarian cancer cell line. Epoxycytochalasin H-treated cells displayed endoplasmic reticulum (ER) stress/unfolded protein response (UPR), diminished mitochondrial membrane potential, mitochondrial injury, and

exacerbated mitophagy and autophagy. Consequently, ovarian cancer cells activated mitochondrial- and ER stress-dependent apoptosis, which led to a reduction in cancer cell proliferation (Wang et al., 2020).

3.5 Autophagy and mitophagy regulate metabolism, and *vice versa*

Autophagy and, particularly, mitophagy are tightly controlled by metabolic cues and, on its turn, provide a layer of metabolic regulation (Deretic and Kroemer, 2022; Palikaras et al., 2015) (recent publications regarding this topic are described below and summarized in Table 3). In addition, many published inducers of canonical autophagy and mitophagy are also activators of a noncanonical autophagy pathway that triggers LC3 lipidation on endo-lysosomal membranes, further supporting the idea that all these cellular mechanisms are interlinked (Jacquin et al., 2017).

Since Beclin1 has been involved in autophagy as well as mitophagy, Son et al. examined the adipose tissue of tamoxifen-induced adipocyte-specific *Beclin1* knock out mice. Three days after the final tamoxifen dose, these mice displayed a hypertrophic enlargement of lipid droplets in brown adipose tissue. In addition, lipolysis/lipid mobilization and energy expenditure induced by treatment with the β3 adrenergic receptor agonist CL316243 was abrogated in these knock out murine model. In contrast, two weeks after the tamoxifen-induced deletion, the animals exhibited reduced expression of lipid metabolism-related genes, low mitochondrial content, necroptosis and apoptosis, macrophage recruitment and inflammation, and defective autophagy/mitophagy in brown adipose tissue. This study demonstrates a link between Beclin1 (a molecule of the autophagy machinery) and energy regulation (particularly lipid metabolism) (Son et al., 2020). Moreover, Ko et al. analyzed mice carrying adipocyte-specific deletion of *Pink1*, a mitophagy-related gene, and found that these mice displayed altered brown adipose tissue function and were obesity-prone. The phenotype was partly due to NLRP3 activation in brown adipocyte precursors, which led to aberrant differentiation into white-like adipocytes. Overall, the authors unveiled a novel mitochondria-NLRP3 pathway that promotes brown adipose tissue dysfunction. Interestingly, data from adipose tissue-specific *Atg7* knock out mice suggest that the role of mitophagy in adipose tissue regulation is different from that of general autophagy (Ko et al., 2021).

The possible role of specific factors, such as the transcription factor EB (TFEB), in autophagic processes have been studied. TFEB is a key regulator

Table 3 Recent studies on the role of autophagy and mitophagy in metabolism.

Topic	Model	Main findings	Reference
Lipid metabolism in brown adipose tissue	*Beclin1*$^{fl/fl}$- *Adipoq-CreER* mice on a C57/BL6 background	Deletion of Beclin1 induces alterations in brown adipose tissue, including defective autophagy/mitophagy	Son et al. (2020)
Adipose tissue differentiation	A variety of knock out mice on a C57BL/6 background	Deletion of the mitophagy-related gene *pink1* affects brown adipose tissue function via NLRP3 activation in brown adipocyte precursors, which leads to aberrant differentiation into white-like adipocytes	Ko et al. (2021)
Lipid metabolism in cardiomyocytes	*TFEB*$^{fl/fl}$- *MHC-Cre* mice on a C57/BL6 background	Nutrient overload-promoted lipid droplet accumulation and caspase-3 activation in cardiomyocytes are enhanced by inhibition of transcription factor EB (TFEB), and alleviated upon overexpression of TFEB	Trivedi et al. (2020)
Aspirin as a caloric restriction mimetic	Multiple mice and *Caenorhabditis elegans* strains	Aspirin inhibits EP300 acetyltransferase, thus epistatically activating autophagy, promoting cardioprotective mitophagy, and mimicking caloric restriction	Pietrocola et al. (2018)
Metabolic syndrome	Primary horse hepatic progenitor cells (HPCs)	Inhibition of low molecular weight protein tyrosine phosphatase (LMPTP) on a cell culture model of metabolic syndrome leads to upregulated expression of autophagy- and mitophagy-related genes, along with reduced expression of UPR-related genes	Alicka et al. (2019)

of lysosomal biogenesis and function. Unexpectedly, when transcriptomic analysis was performed in murine *Tfeb* knock out cardiomyocytes, only a small percentage of the differentially expressed genes were genes related to lysosome function, autophagy and mitophagy. Moreover, nutrient overload-promoted lipid droplet accumulation and caspase-3 activation in cardiomyocytes were affected by TFEB levels. Indeed, these processes were enhanced by inhibition of TFEB and alleviated upon overexpression of TFEB, and these changes persisted upon *Atg7* loss-of-function, indicating that they may be independent of autophagy. Nonetheless, the authors did not further explore the alterations in autophagy and mitophagy in murine TFEB knock out cardiomyocytes (Trivedi et al., 2020).

Of note, salicylate, a metabolite of the commonly used drug aspirin, inhibits EP300 acetyltransferase, thus epistatically activating autophagy, promoting cardioprotective mitophagy, and mimicking caloric restriction in mice and the nematode *Caenorhabditis elegans* (Pietrocola et al., 2018).

Studies from other animals, such as horses, also have shed light to the interplay between mitophagy, autophagy and metabolism. Alicka et al. isolated equine hepatic progenitor-like cells and treated them with palmitate *in vitro* to induce insulin resistance, a key feature of metabolic syndrome. Then, they inhibited low molecular weight protein tyrosine phosphatase (LMPTP) on these cell culture model and observed upregulated expression of autophagy- and mitophagy-related genes, along with reduced expression of UPR-related genes, supporting a role of LMPTP in controlling metabolic homeostasis (Alicka et al., 2019).

4. Conclusions

During the last decades, seminal studies have highlighted the importance of selective autophagy for cellular and organismal homeostasis under physiological and pathological conditions. Nonetheless, the mechanisms that target the autophagic cargo for degradation during selective autophagy are only partially elucidated. New molecular components are being discovered and additional, multifaceted roles emerge for the established ones. Intricate regulatory networks, comprised of overlapping sets of proteins, function in parallel to orchestrate the autophagic response. The core molecular machinery is shared among all types of selective autophagy, yet there are unique receptors that participate in a subset of these pathways, such as xenophagy and mitophagy. Furthermore, distinct cargos of the same type,

such as different bacterial species, or mitochondrial sub-populations (damaged vs superfluous) have been shown to recruit unique receptors and regulatory proteins to mediate their clearance. Several lines of evidence support that autophagy receptors perform non-redundant functions and act on a cooperative fashion to regulate autophagy (Deretic and Kroemer, 2022; Yu et al., 2018).

An evolutionary link between autophagy of bacteria (xenophagy) and autophagy of mitochondria (mitophagy) has been suggested. Mitochondria can be viewed as bacteria-derived endosymbionts and despite the extensive changes that happened to secure the transformation from an independent cell to an integrated organelle of eukaryotic cells, they retain several features of their bacterial origin. These include but are not limited to their structure, method of replication, DNA conformation and similar molecular machineries, such as ribosomes and DNA repair systems (Boguszewska et al., 2020; Meyer et al., 2018a). Not surprisingly, autophagy of bacteria and mitochondria share common molecular components (Randow and Youle, 2014). It has been hypothesized that mitochondrial dysfunction may be perceived by the cell as a result of pathogenic invasion, and thus serves as a signal to activate diverse innate immune response pathways (e.g. the NLRP3-inflammasome) and even initiate xenophagic responses (Manzanillo et al., 2013).

Pharmacological modulation of both non-selective (macro)autophagy and mitophagy has been posed as a promising strategy for many diseases, ranging from infectious diseases to cancer (Vakifahmetoglu-Norberg et al., 2015). Many anti-cancer drugs have cytoprotective autophagy effects that contribute to drug resistance. In order to bypass these side-effects, it has been recently proposed to combine autophagy inducers and inhibitors for cancer treatment (Liu et al., 2020). Oppositely, stimulating mitophagy seems to be a promising strategy to inhibit colon cancer proliferation and attenuate pathological features of Alzheimer's disease (Boyle et al., 2018; Cen et al., 2020). In the case of infectious diseases, activators of host autophagy have been proposed as potential therapies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Maity and Saha, 2021). On the contrary, inhibitors of (macro)autophagy and mitophagy have been successfully tested for the management of plant fungal diseases. Pharmacological inhibition of (macro)autophagy and mitophagy can independently decrease pathogenicity of the fungal phytopathogen *Fusarium graminearum* (Chai et al., 2022). In addition, therapeutic strategies combining anti-bacterial drugs with mitophagy inhibitors, may

show increased efficacy. The nature of the pathogen and the molecular pathways that it employs to invade and multiply into the host have to be considered when generating drugs targeting specific molecules.

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Declarations

Ethical approval and consent to participate: This review article does not involve animals or patients, so this section does not apply.

Consent for publication: The authors give their consent for the publication of this manuscript.

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