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Mitochondria and cell signalling

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Abstract

Mitochondria have long been considered as crucial organelles, primarily for their roles in biosynthetic reactions such as ATP synthesis. However, it is becoming increasingly apparent that mitochondria are intimately involved in cell signalling pathways. Mitochondria perform various signalling functions, serving as platforms to initiate cell signalling, as well as acting as transducers and effectors in multiple processes. Here, we discuss the active roles that mitochondria have in cell death signalling, innate immunity and autophagy. Common themes of mitochondrial regulation emerge from these diverse but interconnected processes. These include: the outer mitochondrial membrane serving as a major signalling platform, and regulation of cell signalling through mitochondrial dynamics and by mitochondrial metabolites, including ATP and reactive oxygen species. Importantly, defects in mitochondrial control of cell signalling and in the regulation of mitochondrial homeostasis might underpin many diseases, in particular age-related pathologies.

Key words: Autophagy, Cell death, Cell signalling, Innate immunity, Mitochondria

Introduction

Mitochondria are often termed the ‘powerhouse’ of the cell for good reason. Not only do mitochondria have a key role in ATP synthesis, but they are also crucial for various other cellular processes, including fatty acid synthesis, Ca^{2+} homeostasis and the biogenesis of haem and iron-sulphur proteins. Given this plethora of functions, it is perhaps not surprising that mitochondria are also heavily integrated into the cell signalling circuitry. The traditional view of mitochondria in signalling is that they represent signalling effectors, for example, by enabling the upregulation of ATP synthesis in response to growth-promoting stimuli. However, more recent evidence demonstrates that mitochondria also actively partici-

pate in numerous biological processes by acting as initiators and transducers of cell signalling. In general, mitochondria regulate cell signalling through two means: serving as physical platforms on which protein–protein signalling interactions occur, and by regulating the levels of intracellular signalling molecules, including Ca^{2+} and reactive oxygen species (ROS). Consequently, mitochondria have been implicated in the regulation of various processes, including growth factor signalling, differentiation and hypoxic stress responses, which are discussed elsewhere (Antico Arciuch et al., 2012; Chandel, 2010; Finkel, 2011; Gunter et al., 2004). In this Commentary, we focus on the signalling roles that mitochondria have in cell death, innate immunity and autophagy. These areas are collectively discussed because of their numerous interconnections. For example, cell death acts as a first-line defence against invading pathogens and serves to alert the innate immune system to infection, whereas autophagy generally acts as a pro-survival mechanism and controls innate immunity at multiple levels, such as through the regulation of pro-inflammatory cytokine production.

Mitochondrial regulation of cell death signalling

Programmed cell death is required for proper development and tissue homeostasis in all multicellular organisms, and its deregulation contributes to various diseases including cancer and neurodegeneration. The predominant form of programmed cell death is apoptosis, a process that requires activation of the caspase proteases. Once activated, caspases cleave several hundred different proteins, leading to rapid apoptotic cell death (Taylor et al., 2008). This is associated with characteristic morphological changes, including plasma membrane blebbing and nuclear condensation. Mitochondria are involved in regulating caspase activity and apoptosis in all multicellular organisms to varying degrees and through different mechanisms (Oberst et al., 2008). For example, in the nematode *Caenorhabditis elegans*, mitochondria serve a non-essential role during apoptosis because, even though they act as a platform for key apoptotic signalling proteins, these proteins do not have to be localised to mitochondria per se in order to regulate apoptosis (Tan et al., 2007). In other organisms, mitochondria have a much more active and important role in apoptosis. Here, we will review how mitochondria actively contribute to apoptotic cell death and discuss their potential roles in other, non-apoptotic, forms of programmed cell death.

Mitochondrial outer membrane permeabilisation and apoptosis

Within the intermembrane space, mitochondria sequester various proteins, such as cytochrome *c*, that directly activate caspases following their release into the cytoplasm (Tait and Green, 2010). Consequently, the integrity of the outer mitochondrial membrane (OMM) is strictly regulated through interactions between pro- and anti-apoptotic members of the BCL2 protein family (Chipuk et al., 2010). In the intrinsic or mitochondrial pathway of apoptosis, pro-apoptotic insults such as DNA damage lead to the activation of two key BCL2 family proteins, BAX and BAK. Active BAX and BAK cause mitochondrial outer membrane permeabilisation (MOMP), enabling the release of cytochrome *c* and SMAC (also known as DIABLO) into the cytoplasm where they promote activation of caspases (Fig. 1). Provided MOMP has occurred, cells can also undergo caspase-independent cell death that is most likely to be a consequence of a progressive decline in mitochondrial function (Lartigue et al., 2009; Tait and Green, 2008). The absolute requirement for mitochondria in intrinsic apoptosis is best demonstrated by two findings: cells deficient in both BAX and BAK are resistant to all intrinsic apoptotic

stimuli, and cells expressing a point mutant of cytochrome *c* (K72A), with a reduced ability to activate caspases but that retains respiratory-chain function, fail to efficiently activate caspases following MOMP, indicating that mitochondria, through MOMP, are required for caspase activation and apoptosis (Hao et al., 2005; Wei et al., 2001).

Although MOMP is most closely associated with the execution of apoptosis, under some circumstances it can also be involved in non-lethal signalling functions. For example, it has been suggested that mitochondrial activation of caspase-3 is required for effective internalisation of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor at post-synaptic membranes and that mitochondrial activation of caspase-9 regulates myocyte differentiation in vitro (Li et al., 2010; Murray et al., 2008). Interestingly, MOMP can be incomplete, allowing some mitochondria to remain intact (Tait et al., 2010); this incomplete MOMP is probably essential for non-cytotoxic signalling functions of MOMP if the cell is to survive. Incomplete MOMP occurs, at least in part, through two mechanisms. In the first, mitochondria that evade MOMP typically express higher levels of anti-apoptotic BCL2 proteins (Tait et al., 2010). The second mechanism relates to mitochondrial dynamics. Mitochondria are constantly undergoing rounds of fission and fusion with one another (Westermann, 2010). Inhibition of mitochondrial fusion promotes incomplete MOMP, probably by preventing MOMP of one mitochondrion from causing MOMP in other mitochondria (as would occur if mitochondria were fused). Other potential mechanisms that cause incomplete MOMP, particularly where MOMP occurs in a minority of mitochondria, might include localised activation of BH3-only proteins – the family of proteins that trigger BAX and BAK activation and MOMP.

Mitochondrial regulation of caspase-8 activity

Recent findings suggest that mitochondria also regulate apoptosis through a means other than MOMP by serving as a platform to regulate caspase-8 activation. In the extrinsic pathway of apoptosis, following ligand binding, death receptors induce apoptosis through a process that requires activation of caspase-8 at the intracellular tail of the receptor–ligand complex (Fig. 1) (Kaufmann et al., 2011). In a cell-type-dependent manner, active caspase-8 can either directly activate executioner caspases and cause apoptosis (in type I cells) or, alternatively, require MOMP for effective executioner caspase activation and apoptosis (in type II cells) (Scaffidi et al., 1998). Caspase-8 induces MOMP through cleavage and activation of the pro-apoptotic BCL2 family protein BID, which, in turn, activates BAX and BAK (Fig. 1) (Li et al., 1998; Luo et al., 1998). In type II cells, MOMP promotes caspase activation by causing the release of mitochondrial proteins, including SMAC, that block the ability of XIAP (X-linked inhibitor of apoptosis) to inhibit caspase function (Jost et al., 2009). Interestingly, mitochondria have recently been shown to be required for effective initiator caspase-8 activity in type II cells following death receptor ligation (Gonzalvez et al., 2008). In type II cells, caspase-8 is recruited and activated at the OMM in a process that appears to be dependent upon the mitochondrial membrane phospholipid cardiolipin. Mitochondrially localised caspase-8 complexes with and cleaves BID, leading to the induction of MOMP (Schug et al., 2011). This is functionally important because disruption of the mitochondrial association of caspase-8 inhibits both caspase-8 activity and subsequent MOMP that is required for apoptosis in type II cells (Gonzalvez et al., 2008). In this setting, the OMM serves as a signalling platform, both facilitating and focusing caspase-8 activity to where it is required. Several important questions remain. For example, how is caspase-8 targeted to the mitochondria? And what contribution,

if any, does caspase-8 that is localised to the mitochondria have in the extrinsic death-receptor-mediated death of type I cells?

Roles for mitochondria in non-apoptotic cell death

Mitochondria might have a role in other forms of programmed cell death that exist besides apoptosis (Galluzzi et al., 2011). One important example is a necrosis-like cell death pathway that is activated by various triggers, including death-receptor ligation, which requires the kinase receptor-interacting serine/threonine protein kinase 3 (RIPK3) (Vandenabeele et al., 2010). This RIPK3-dependent necrosis has numerous important roles in vivo during embryonic development and in host antiviral immunity (Kaiser et al., 2011; Oberst et al., 2011; Upton et al., 2010; Zhang et al., 2011). How RIPK3 activity kills cells remains unclear, although several lines of evidence suggest that mitochondria have a role for in this process. For example, during RIPK3-dependent necrosis, increases in cellular ROS can be detected and ROS scavengers can offer protection against death, indicating that ROS might be required for RIPK3-dependent necrosis (Schulze-Osthoff et al., 1992; Zhang et al., 2009a). As mitochondria represent a major cellular source of ROS, this suggests that they are central to the execution of the RIPK3-dependent necrosis pathway. However, it remains unclear whether mitochondrially generated ROS are required for RIPK3-dependent necrosis for two reasons: first, besides mitochondria, other cellular sources of ROS exist, and second, some studies have found that ROS scavengers have no protective effect (Festjens et al., 2006; He et al., 2009).

An alternative role for mitochondria in RIPK3-dependent necrosis centres upon the rapid depletion of cellular ATP that is observed during death, which could be causal in the death process (Temkin et al., 2006). Along these lines, one study found that the interaction of adenine nucleotide transporter (ANT) with cyclophilin D (CYPD) is inhibited during RIPK3-dependent necrosis, leading to a reduction of ADP and ATP transport across the mitochondria and a lowering of cellular ATP levels (Temkin et al., 2006). However, recent data demonstrate that RIPK3-dependent necrosis is unaffected by the absence of CYPD, thereby arguing against a crucial role for an ANT–CYPD interaction in this process (Ch'en et al., 2011). In contrast to RIPK3-dependent necrosis, CYPD appears to be required for necrosis that is triggered by ROS and Ca^{2+} overload, implying that there is a key role for mitochondria in necrosis triggered by these stimuli (Baines et al., 2005; Nakagawa et al., 2005). In summary, mitochondria clearly have essential roles in apoptotic cell death and in some types of necrosis. Determining potential roles for mitochondria in other forms of cell death will be facilitated through further understanding of the molecular mechanisms that execute non-apoptotic cell death and determining whether loss of mitochondrial function(s) impacts on any given form of cell death.

Mitochondrial regulation of innate immunity

Innate immunity forms an evolutionarily conserved front-line defence against microbial invasion. Host cells of the innate immune system, such as macrophages, detect infectious microorganisms or damaged cells through pattern recognition receptors (PRRs) (Medzhitov, 2007). PRRs recognise conserved molecular patterns that are shared by different microorganisms [pathogen-associated molecular patterns (PAMPs)] or proteins that are released from damaged cells [damage-associated molecular patterns (DAMPs)]. Upon activation, PRRs elicit the production of various pro-inflammatory cytokines, type I

interferons (IFNs) and co-stimulatory molecules. Ultimately, this creates a potent anti-microbial milieu and, in higher organisms, ensures the appropriate activation of the adaptive immune response. Mounting evidence demonstrates that mitochondria regulate innate immunity at multiple levels, including mitochondria serving as DAMPs themselves, forming platforms for downstream signalling and responding as effectors, primarily through the generation of ROS, to facilitate the anti-microbial host-cell response (West et al., 2011b). Here, we will discuss recent findings that perhaps best highlight mitochondrial regulation of innate immunity by focusing on how mitochondria control antiviral innate immunity, inflammasome activity and the innate effector response.

Mitochondria and antiviral immunity

A key class of PRRs that respond to viral infection is the retinoic-acid-inducible protein I (RIG-I, also known as DDX58)-like receptor (RLR) family, which comprises three members: RIG-I, MDA5 (also known as IFIH1) and DHX58. RLRs function in antiviral immunity by detecting viral cytoplasmic double-stranded RNA (dsRNA) and activating the production of type I IFNs and pro-inflammatory cytokines (Fig. 2). A role for mitochondria in RLR-activated antiviral immunity was first established through the identification of the RLR mitochondrial adaptor protein MAVS (mitochondrial antiviral-signalling protein) (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Xu et al., 2005). MAVS localises to the OMM through its C-terminal transmembrane domain, which has similarities to other OMM-localised proteins such as BCL-XL (the long isoform of the protein encoded by *BCL2L1*) (Seth et al., 2005). Importantly, localisation of MAVS to the OMM is crucial for it to function as an adaptor for RIG-I- and MDA5-mediated antiviral signalling (Seth et al., 2005). Following recognition of viral dsRNA, RIG-I and MDA5 might interact with MAVS through mutual caspase activation and recruitment domains (CARDs), although formal proof of this interaction occurring in vivo is lacking. Upon RIG-I activation, MAVS displays prion-like properties, whereby activated MAVS can self-propagate the activation signal and activate other MAVS molecules (Fig. 2) (Hou et al., 2011). The accumulation of aggregated MAVS on the OMM leads to activation of downstream pathways such as interferon regulatory factor 3 (IRF3) and nuclear factor-κB (NF-κB) through the ability of activated MAVS to bind downstream signalling components, including TRADD and TNF receptor-associated factor 6 (TRAF6) (West et al., 2011b). Additional mitochondrial proteins regulate the RIG-I–MAVS pathway, including positive regulators such as TOM70 and STING (also known as TMEM173), whereas negative regulators include NLRX1 (Liu et al., 2010; Moore et al., 2008; Tattoli et al., 2008). Interestingly, STING is required for RLR signalling but localises to endoplasmic reticulum (ER)–mitochondrial contact sites (Ishikawa and Barber, 2008; Zhong et al., 2008). Collectively, these findings argue that the OMM has a crucial role as a signalling platform for antiviral RLR signalling.

Recent findings also demonstrate a central role for mitochondrial dynamics in the control of antiviral RLR signalling. Although mitochondrial dynamics probably evolved to maintain mitochondrial homeostasis, it is becoming increasingly apparent that mitochondrial fission and fusion control other processes, including antiviral RLR signalling. At the molecular level, mitochondrial fusion is positively regulated by mitofusin proteins (MFN1 and MFN2) and negatively regulated by the fission-promoting proteins DRP1 and FIS1 (Castanier et al., 2010; Onoguchi et al., 2010; Yasukawa et al., 2009). Interestingly, MFN2 interacts with MAVS and its overexpression inhibits RLR signalling (Fig. 2); however, this inhibitory effect might not simply be due to its effects on mitochondrial dynamics per se, as

overexpression of MFN1 has no effect on RLR signalling (Yasukawa et al., 2009). This specific effect of MFN2 might relate to its recently described key role in regulating mitochondrial–ER contact sites, perhaps by affecting the ability of STING to positively regulate RLR signalling (de Brito and Scorrano, 2008). Several additional studies have demonstrated that mitochondrial fusion promotes RLR signalling, whereas fission inhibits it, although it remains unclear why mitochondrial dynamics influence antiviral signalling so profoundly. Conceivably, it might be due to the prion-like self-activation of MAVS molecules following the initial activating stimulus – here, a continuous, fused mitochondrial network might promote MAVS self-activation and robust antiviral signalling, whereas this would be inhibited when mitochondria had undergone excessive fission.

Mitochondria, DAMPs and inflammasome activation

The innate immune system responds to host cellular damage by recognising DAMPs (Chen and Nunez, 2010). Typically, DAMPs are endogenous intracellular molecules that are released from cells following damage, for example, from cells undergoing necrotic cell death. DAMPs effectively initiate signalling cascades in innate immune cells, such as macrophages, thereby leading to upregulation of pro-inflammatory cytokines, type I IFNs and co-stimulatory molecules. In response, the innate immune system both promotes resolution of tissue injury and enhances immune surveillance at the site of damage.

Mitochondria represent a rich source of DAMPs, a characteristic that probably stems from their bacterial ancestry (Medzhitov, 2007). Various studies have demonstrated that mitochondrial DNA acts as an effective DAMP. For example, direct injection of mitochondrial (but not nuclear) DNA into mouse joints induces a pro-inflammatory response (Collins et al., 2004). The ability of mitochondrial DNA to serve as a DAMP probably relates to its similarity to bacterial DNA, in that both share hypomethylated CpG motifs that are required for the effective activation of the PRR Toll-like receptor 9 (TLR9). Interestingly, recent studies have demonstrated that mitochondrial DNA is released systemically during trauma and that injection of mitochondria lysates containing mitochondrial DNA induces lung and liver inflammation (Zhang et al., 2010). In that study, it was proposed that mitochondrial DAMPs drive hyperactivation of innate immunity, which might underlie systemic inflammatory response syndrome, a syndrome that clinically resembles shock but can occur in a sterile environment.

Mitochondria and bacterial protein synthesis both use *N*-formyl-methionine as the initiating residue. Bacterial *N*-formyl peptides serve as PAMPs and are recognised by G-protein-coupled formyl peptide receptors (FPRs) (Rabiet et al., 2007). Along similar lines, mitochondrial *N*-formyl peptides have been found to represent effective DAMPs, and they act through the receptor FPR-1 to stimulate neutrophil chemotaxis and cytokine secretion (Carp, 1982; Zhang et al., 2010). Mitochondrial DNA and N-formyl peptides represent two sources of mitochondrial DAMPs, but others, including mitochondrially generated ATP, can also fulfil this function. As we will now discuss, recent data also demonstrate a key role for mitochondrial ROS as DAMPs that activate the NLRP3 (NLR family, pyrin domain-containing 3) inflammasome.

Cytoplasmic PRRs include certain members of the nucleotide oligomerisation domain (NOD)-like receptor family that, upon activation, form multi-subunit protein complexes termed inflammasomes (Schroder and Tschopp, 2010). Mature inflammasomes activate caspase-1, leading to proteolytic cleav-

age and maturation of the pro-inflammatory cytokine interleukin 1β (IL-1β). Two recent studies have found that mitochondria have a central role in controlling the activation of the NLRP3 inflammasome (Fig. 3) (Nakahira et al., 2011; Zhou et al., 2011). Diverse PAMPs and DAMPs such as lipopolysaccharides (LPSs), asbestos and uric acid lead to NLRP3 activation through upregulation of ROS (Dostert et al., 2008). Although many cellular sites of ROS generation exist, mitochondrially generated ROS appear to be key for activating the NLRP3 inflammasome. Inhibition of mitochondrial ROS generation effectively blocks the NLRP3 activation after various stimuli, whereas increased mitochondrial ROS (caused through inhibition of oxidative phosphorylation) is sufficient to trigger NLRP3 activity (Zhou et al., 2011). Interestingly, blocking autophagic removal of defective mitochondria leads to upregulation of ROS and NLRP3-driven IL-1β release, consistent with earlier findings demonstrating excessive release of IL-1β in autophagy-deficient macrophages in response to LPS (Nakahira et al., 2011; Saitoh et al., 2008; Zhou et al., 2011). Besides mitochondrial ROS, release of mitochondrial DNA into the cytoplasm has also been shown to facilitate NLRP3 activation (Fig. 3) (Nakahira et al., 2011). Although these findings demonstrate a crucial role for mitochondria in activating the NLRP3 inflammasome, many questions remain. Not least, it is unclear exactly how mitochondrial ROS activate NLRP3, and whether this is activation is direct or indirect. Nor is it understood how diverse PAMPs and DAMPs converge on the mitochondria to effect ROS production, although, as we will discuss below, some mechanisms are being uncovered. Finally, given that mitochondrial DNA facilitates NLRP3 activation, it will be of interest to determine the mechanism whereby mitochondrial DNA can be released from mitochondria without invoking the release of pro-apoptotic intermembrane-space proteins such as cytochrome *c*.

Mitochondrial signalling and the innate effector response

A mainstay of innate immunity is the effective phagocytosis and destruction of invading bacteria. During phagocytosis, a respiratory burst ensues, leading to massive upregulation of ROS, which are required for killing the phagocytosed microorganism. Although the majority of ROS production during phagocytosis occurs through NAPDH oxidases, emerging evidence demonstrates that mitochondrial ROS also have a key role (West et al., 2011b). As we have discussed, mitochondrial ROS act as signalling molecules in innate immunity, but recent data demonstrate that regulated generation of mitochondrial ROS is also important for antimicrobial activity during phagocytosis (Fig. 3) (West et al., 2011a). Engagement of certain TLRs (TLR1, 2 and 4) leads to mitochondrial translocation of the signalling adaptor TRAF6. At the mitochondria, TRAF6 interacts with ECSIT, a protein that has been implicated in mitochondrial respiratory complex I assembly (a key component of the oxidative phosphorylation system). In turn, TRAF6-dependent ubiquitylation of ECSIT leads to increased mitochondrial ROS facilitating destruction of phagocytosed bacteria (Fig. 3). Although further elucidation of how ECSIT regulates mitochondrial ROS production is clearly required, the study highlights the integral effector role that mitochondria have in innate immune signalling pathways.

Mitochondrial regulation of autophagy and mitophagy

Macroautophagy, hereafter termed autophagy, is an evolutionarily conserved lysosome-dependent degradation process (Mizushima et al., 2008). During autophagy, cytosolic cargo, including proteins and organelles, are engulfed by a double-membraned lipid bilayer that seals to form the autophago-

some. The mature autophagosome fuses with lysosomes, leading to destruction and recycling of the engulfed cargo. Autophagy, therefore, both acts as a cellular detoxification mechanism and produces metabolites for energy production and anabolic processes. Basal levels of autophagy occur in all cells, however, under stressful conditions such as nutrient depletion, autophagy is highly upregulated, thereby providing an alternate source of metabolites for energy production (Mizushima et al., 2008). Given this, it is perhaps unsurprising that autophagy has crucial functions in numerous aspects of health and disease (Mizushima et al., 2008). Mounting evidence points to key signalling roles for mitochondrial regulation of autophagy at various stages (Fig. 4). These include mitochondrial regulation of the signalling processes that initiate autophagy and mitochondria themselves serving as a membrane source for autophagosome formation. Here, we will review how mitochondria regulate the multistep process of autophagy, and discuss recent findings addressing how damaged mitochondria signal their dysfunction and, in doing so, promote their removal through a selective form of autophagy termed mitophagy.

Mitochondrial regulation of autophagy

In mammalian cells, under most circumstances, the kinases ULK1 or ULK2 must be activated in order to initiate autophagy (Mizushima, 2010). ULK1 resides in a complex with FIP200 (also known as RB1CC1) and ATG13 that, following activation, initiates nucleation and elongation of the autophagosome. ULK1 activity is therefore highly regulated, primarily through phosphorylation by upstream kinases. Under nutrient-replete conditions, the mammalian target of rapamycin complex 1 (mTORC1) kinase complex is activated, leading to inhibitory phosphorylation of ULK1 and ATG13, thereby repressing autophagy (Zoncu et al., 2011). Following nutrient starvation, reduction of cellular ATP, which is largely produced by mitochondria, leads to activation of AMP-activated protein kinase (AMPK), which, in turn, activates ULK1 directly by phosphorylation and indirectly by inhibitory phosphorylations of the mTORC1 regulator TSC2 and RAPTOR (a component of the mTORC1 complex) (Fig. 4) (Egan et al., 2011; Inoki et al., 2003; Kim et al., 2011). Consequently, mitochondria, through their ability to generate ATP, and regulate ADP and ATP levels, regulate autophagy.

Recent data also demonstrate that mitochondria have a crucial role in the regulation of autophagy mediated by intracellular Ca^{2+} (Cardenas et al., 2010). Mitochondria take up Ca^{2+} that is released by the ER upon activation of inositol triphosphate receptors. In turn, Ca^{2+} uptake by mitochondria supports efficient oxidative phosphorylation, ATP generation and inhibition of autophagy through repression of AMPK activity. Interestingly, besides ATP, mitochondria also regulate the initiation of autophagy through the production of ammonia generated by mitochondrial-dependent glutaminolysis (Eng et al., 2010). Ammonia upregulates autophagy in a non-conventional manner, as it does not require either ULK1 or ULK2 (Fig. 4) (Cheong et al., 2011). The ability of ammonia to upregulate autophagy might be important in cancer, as cancer cells typically display high levels of glutaminolysis, and autophagy appears to be crucial to tumourigenesis, at least in some settings (Guo et al., 2011; Yang et al., 2011). Finally, a recent study has found that, in yeast, mitochondria are absolutely required to initiate autophagy by regulating the activity of the protein kinase A (PKA) (Graef and Nunnari, 2011). In this case, it was observed that mitochondrial respiratory deficiency led to upregulation of PKA activity, which inhibited autophagy in two ways: by blocking Atg1–Atg13 complex activity (the yeast equivalent of the ULK1–ATG13–FIP200 complex) and by inhibiting the expression of Atg8 [microtubule-associated protein light chain 3 (LC3) in mammals], which is required for autophagy (Fig. 4).

It has been difficult to unambiguously determine the membrane source for autophagosome formation in mammalian cells (Chen and Klionsky, 2011; Tooze and Yoshimori, 2010). Indeed, it is probable that various membrane compartments, including the ER and plasma membrane, can serve this purpose (Hayashi-Nishino et al., 2010; Ravikumar et al., 2010). Recently, mitochondria have been demonstrated to serve as the membrane source for autophagosome formation specifically following starvation (Fig. 4) (Hailey et al., 2010). In that study, the authors found that nascent autophagosomes formed from lipid derived from the OMM. Autophagosome elongation requires conjugation of LC3 (Atg8 in yeast) to the lipid phosphatidylethanolamine (PtdEtn). Mitochondria generate PtdEtn from phosphatidylserine (PtdSer) that is obtained from the ER through ER–mitochondria contact sites. Consequently, disruption of these contact sites was found to inhibit autophagosome formation following starvation. It is unclear exactly why mitochondria contribute to autophagosome formation specifically following starvation. One scenario is that PtdEtn generation in the ER is inhibited upon starvation, and therefore mitochondria represent the only source of PtdEtn under this condition. An alternative possibility might relate to the finding that a key protein involved in autophagosome nucleation, beclin-1, binds anti-apoptotic BCL2 proteins that reside, at least in part, on the OMM (Liang et al., 1998). The interaction of beclin-1 with BCL2 effectively inhibits the activity of beclin-1 and autophagy (Pattingre et al., 2005). Upon starvation, JNK-mediated phosphorylation of BCL2 disrupts the BCL2–beclin-1 interaction, allowing autophagy to proceed (Wei et al., 2008). Perhaps following starvation, this interaction is specifically disrupted on the OMM, allowing it to serve as a source of autophagosome nucleation and biogenesis.

Mechanisms and functions of mitophagy

Under some circumstances, autophagy can selectively target organelles or proteins for degradation (Wang and Klionsky, 2011). Recently, several pathways leading to specific autophagic degradation of mitochondria, termed mitophagy, have been described (Youle and Narendra, 2011). Through its ability to remove damaged mitochondria, mitophagy acts to maintain a healthy population of mitochondria. Mitophagy is also important during erythropoiesis, as depletion of mitochondria is required during reticulocyte-to-erythrocyte maturation (Schweers et al., 2007; Sandoval et al., 2008). Here, we discuss the predominant mechanisms of mitophagy in mammalian cells, focusing on the PINK1–Parkin pathway, NIX-mediated mitophagy and a newly described form of mitophagy that is induced by an ATG12–ATG3 complex. It should be noted that additional, apparently non-conserved, mitophagic signalling pathways exist in other organisms such as yeast (Kanki et al., 2009; Okamoto et al., 2009).

Recent work implicates defective mitophagy in Parkinson's disease (Narendra et al., 2008). In some cases of autosomal-recessive Parkinson's disease, the gene encoding Parkin (*PARK2*), a cytosolic E3 ubiquitin ligase, is mutated, leading to loss of function (Kitada et al., 1998). Recently, Parkin has been found to translocate to dysfunctional mitochondria where it promotes mitophagy (Fig. 5) (Narendra et al., 2008; Geisler et al., 2010; Vives-Bauza et al., 2010; Matsuda et al., 2010). Mitochondrial recruitment of Parkin is dependent on the mitochondrial kinase PINK1, which, similar to Parkin, is mutated in some cases of juvenile-onset Parkinson's disease (Geisler et al., 2010; Matsuda et al., 2010; Narendra et al., 2010; Vives-Bauza et al., 2010). PINK1 is normally imported into the mitochondrial intermembrane space, where it is inactivated through cleavage by the rhomboid protease PARL (Narendra et al., 2010). Following loss of mitochondrial membrane potential (signalling unhealthy mitochondria), PINK1 accumulates on the OMM and recruits Parkin in a manner dependent on PINK1 kinase activity.

On the OMM, Parkin, through its function as an E3 ubiquitin ligase, promotes mitophagy that is dependent on the AAA+ ATPase p97 (also known as VCP) (Tanaka et al., 2010). In summary, Parkin can promote specific removal of dysfunctional mitochondria dependent upon its mitochondrial activation by PINK1 and its ubiquitin ligase activity. However, fundamental questions remain: what are the relevant ubiquitination target(s) for Parkin-mediated mitophagy and how does PINK1 kinase activity lead to Parkin activation? Moreover, a major advancement would be to develop a means to monitor mitophagy in vivo as the actual occurrence and importance of Parkin-mediated mitophagy in vivo remains unclear.

Reticulocytes actively remove their intracellular organelles as they mature into erythrocytes. During maturation, there is a wave of programmed mitophagy that effectively depletes all mitochondria from the cell. Recently, the protein NIX (also termed BNIP3) was identified as a key player in this process (Fig. 5) (Sandoval et al., 2008; Schweers et al., 2007). Originally identified as a BH3-only BCL2 family member, NIX expression is strongly upregulated during reticulocyte maturation where it localises to the mitochondria and induces mitophagy. Exactly how NIX induces mitophagy is not understood, but several possibilities exist. These include NIX acting to recruit mitochondria into the maturing autophagosome through its ability to bind the key autophagy protein LC3 (Novak et al., 2010). Alternatively, on the OMM, NIX might displace beclin-1 from anti-apoptotic BCL2 proteins promoting autophagosome formation on the mitochondria. Interestingly, NIX can also induce mitochondrial clearance in cells that are autophagy deficient, albeit with slower kinetics (Zhang et al., 2009b).

In addition to NIX- and Parkin-mediated mitophagy, other pathways exist. For example, mitophagy can be activated in reticulocytes in a NIX-independent manner, either through induction of MOMP or mitochondrial depolarisation (Sandoval et al., 2008). Along these lines, a new mitophagy pathway that requires the autophagy proteins ATG12 and ATG3 has been described (Radoshevich et al., 2010). Normally, ATG12 covalently binds ATG5, a crucial step in autophagy that enables LC3 conjugation to PE. However, in that recent study, ATG12 was found to covalently attach to ATG3. This newly described ATG12–ATG3 complex promotes mitophagy following dissipation of mitochondrial membrane potential, although how it achieves this is unclear. Interestingly, in addition to regulating mitophagy, the ATG12–ATG3 complex also regulates mitochondria homeostasis through inhibition of mitochondrial expansion and promotion of mitochondrial fusion (Radoshevich et al., 2010).

Conclusion and future perspectives

We have focused on the key signalling roles that mitochondria have in cell death, innate immunity and autophagy. Through their ability to regulate key signalling mediators such as ROS, mitochondria are ideally suited to control many signalling processes. Additionally, mitochondria probably control cell signalling through other means; for example, mitochondria produce citrate that serves as the major source of acetyl-CoA for protein acetylation, a key post-translational modification involved in many signalling processes. Emerging data also support crucial, although poorly understood, roles for mitochondrial dynamics and physical interactions between mitochondria and the ER in various signalling pathways. Mitochondrial regulation of these signalling processes is likely to have numerous pathophysiological roles. For example, autophagy, and perhaps mitophagy, decline with age, resulting in increased mitochondrial damage. This impacts on sensitivity to cell death, increased inflammatory cytokine production (through DAMPs and ROS) and, possibly, reduced antiviral responses. Collectively, these

changes that centre on mitochondrial signalling might be instrumental in the processes of aging and cancer.

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Footnotes

This article is part of a Minifocus on Mitochondria. For further reading, please see related articles: 'PINK1- and Parkin-mediated mitophagy at a glance' by Seok Min Jin and Richard J. Youle (*J. Cell Sci.* **125**, 795-799) and 'Mitochondrial redox signalling at a glance' by Yvonne Collins et al. (*J. Cell Sci.* **125**, 801-806).

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References

Antico Arciuch V. G., Elguero M. E., Poderoso J. J., Carreras M. C. (2012). Mitochondrial regulation of cell cycle and proliferation. *Antioxid. Redox Signal.* doi: 10.1089/ars.2011.4085 [PMCID: PMC3315176] [PubMed: 21967640]

Baines C. P., Kaiser R. A., Purcell N. H., Blair N. S., Osinska H., Hambleton M. A., Brunskill E. W., Sayen M. R., Gottlieb R. A., Dorn G. W., et al. (2005). Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* 434, 658-662 [PubMed: 15800627]

Cardenas C., Miller R. A., Smith I., Bui T., Molgo J., Muller M., Vais H., Cheung K. H., Yang J., Parker I., et al. (2010). Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca2+ transfer to mitochondria. *Cell* 142, 270-283 [PMCID: PMC2911450] [PubMed: 20655468]

Carp H. (1982). Mitochondrial N-formylmethionyl proteins as chemoattractants for neutrophils. *J. Exp. Med.* 155, 264-275 [PMCID: PMC2186576] [PubMed: 6274994]

Castanier C., Garcin D., Vazquez A., Arnoult D. (2010). Mitochondrial dynamics regulate the RIG-I-like receptor antiviral pathway. *EMBO Rep.* 11, 133-138 [PMCID: PMC2828750] [PubMed: 20019757]

Ch'en I. L., Tsau J. S., Molkentin J. D., Komatsu M., Hedrick S. M. (2011). Mechanisms of necroptosis in T cells. *J. Exp. Med.* 208, 633-641 [PMCID: PMC3135356] [PubMed: 21402742]

Chandel N. S. (2010). Mitochondrial regulation of oxygen sensing. *Adv. Exp. Med. Biol.* 661, 339-354 [PubMed: 20204741]

Chen G. Y., Nunez G. (2010). Sterile inflammation: sensing and reacting to damage. *Nat. Rev. Immunol.* 10, 826-837 [PMCID: PMC3114424] [PubMed: 21088683]

Chen Y., Klionsky D. J. (2011). The regulation of autophagy-unanswered questions. *J. Cell Sci.* 124, 161-170 [PMCID:

PMC3037094] [PubMed: 21187343]

Cheong H., Lindsten T., Wu J., Lu C., Thompson C. B. (2011). Ammonia-induced autophagy is independent of ULK1/ULK2 kinases. *Proc. Natl. Acad. Sci. USA* 108, 11121-11126 [PMCID: PMC3131371] [PubMed: 21690395]

Chipuk J. E., Moldoveanu T., Llambi F., Parsons M. J., Green D. R. (2010). The BCL-2 family reunion. *Mol. Cell* 37, 299-310 [PMCID: PMC3222298] [PubMed: 20159550]

Collins L. V., Hajizadeh S., Holme E., Jonsson I. M., Tarkowski A. (2004). Endogenously oxidized mitochondrial DNA induces in vivo and in vitro inflammatory responses. *J. Leukoc. Biol.* 75, 995-1000 [PubMed: 14982943]

de Brito O. M., Scorrano L. (2008). Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* 456, 605-610 [PubMed: 19052620]

Dostert C., Petrilli V., Van Bruggen R., Steele C., Mossman B. T., Tschopp J. (2008). Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science* 320, 674-677 [PMCID: PMC2396588] [PubMed: 18403674]

Egan D. F., Shackelford D. B., Mihaylova M. M., Gelino S., Kohnz R. A., Mair W., Vasquez D. S., Joshi A., Gwinn D. M., Taylor R., et al. (2011). Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. *Science* 331, 456-461 [PMCID: PMC3030664] [PubMed: 21205641]

Eng C. H., Yu K., Lucas J., White E., Abraham R. T. (2010). Ammonia derived from glutaminolysis is a diffusible regulator of autophagy. *Sci. Signal.* 3, ra31 [PubMed: 20424262]

Festjens N., Kalai M., Smet J., Meeus A., Van Coster R., Saelens X., Vandenabeele P. (2006). Butylated hydroxyanisole is more than a reactive oxygen species scavenger. *Cell Death Differ.* 13, 166-169 [PubMed: 16138110]

Finkel T. (2011). Signal transduction by reactive oxygen species. *J. Cell Biol.* 194, 7-15 [PMCID: PMC3135394] [PubMed: 21746850]

Galluzzi L., Vitale I., Abrams J. M., Alnemri E. S., Baehrecke E. H., Blagosklonny M. V., Dawson T. M., Dawson V. L., El-Deiry W. S., Fulda S., et al. (2011). Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ.* 19, 107-120 [PMCID: PMC3252826] [PubMed: 21760595]

Geisler S., Holmstrom K. M., Skujat D., Fiesel F. C., Rothfuss O. C., Kahle P. J., Springer W. (2010). PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat. Cell Biol.* 12, 119-131 [PubMed: 20098416]

Gonzalvez F., Schug Z. T., Houtkooper R. H., MacKenzie E. D., Brooks D. G., Wanders R. J., Petit P. X., Vaz F. M., Gottlieb E. (2008). Cardiolipin provides an essential activating platform for caspase-8 on mitochondria. *J. Cell Biol.* 183, 681-696 [PMCID: PMC2582890] [PubMed: 19001123]

Graef M., Nunnari J. (2011). Mitochondria regulate autophagy by conserved signalling pathways. *EMBO J.* 30, 2101-2114 [PMCID: PMC3117638] [PubMed: 21468027]

Gunter T. E., Yule D. I., Gunter K. K., Eliseev R. A., Salter J. D. (2004). Calcium and mitochondria. *FEBS Lett.* 567, 96-102 [PubMed: 15165900]

Guo J. Y., Chen H. Y., Mathew R., Fan J., Strohecker A. M., Karsli-Uzunbas G., Kamphorst J. J., Chen G., Lemons J. M., Karantza V., et al. (2011). Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev.* 25, 460-470 [PMCID: PMC3049287] [PubMed: 21317241]

Hailey D. W., Rambold A. S., Satpute-Krishnan P., Mitra K., Sougrat R., Kim P. K., Lippincott-Schwartz J. (2010). Mitochondria

supply membranes for autophagosome biogenesis during starvation. *Cell* 141, 656-667 [PMCID: PMC3059894] [PubMed: 20478256]

Hao Z., Duncan G. S., Chang C. C., Elia A., Fang M., Wakeham A., Okada H., Calzascia T., Jang Y., You-Ten A., et al. (2005). Specific ablation of the apoptotic functions of cytochrome C reveals a differential requirement for cytochrome C and Apaf-1 in apoptosis. *Cell* 121, 579-591 [PubMed: 15907471]

Hayashi-Nishino M., Fujita N., Noda T., Yamaguchi A., Yoshimori T., Yamamoto A. (2010). Electron tomography reveals the endoplasmic reticulum as a membrane source for autophagosome formation. *Autophagy* 6, 301-303 [PubMed: 20104025]

He S., Wang L., Miao L., Wang T., Du F., Zhao L., Wang X. (2009). Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. *Cell* 137, 1100-1011 [PubMed: 19524512]

Hou F., Sun L., Zheng H., Skaug B., Jiang Q. X., Chen Z. J. (2011). MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response. *Cell* 146, 448-461 [PMCID: PMC3179916] [PubMed: 21782231]

Inoki K., Zhu T., Guan K. L. (2003). TSC2 mediates cellular energy response to control cell growth and survival. *Cell* 115, 577-590 [PubMed: 14651849]

Ishikawa H., Barber G. N. (2008). STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature* 455, 674-678 [PMCID: PMC2804933] [PubMed: 18724357]

Jost P. J., Grabow S., Gray D., McKenzie M. D., Nachbur U., Huang D. C., Bouillet P., Thomas H. E., Borner C., Silke J., et al. (2009). XIAP discriminates between type I and type II FAS-induced apoptosis. *Nature* 460, 1035-1039 [PMCID: PMC2956120] [PubMed: 19626005]

Kaiser W. J., Upton J. W., Long A. B., Livingston-Rosanoff D., Daley-Bauer L. P., Hakem R., Caspary T., Mocarski E. S. (2011). RIP3 mediates the embryonic lethality of caspase-8-deficient mice. *Nature* 471, 368-372 [PMCID: PMC3060292] [PubMed: 21368762]

Kanki T., Wang K., Cao Y., Baba M., Klionsky D. J. (2009). Atg32 is a mitochondrial protein that confers selectivity during mitophagy. *Dev. Cell* 17, 98-109 [PMCID: PMC2746076] [PubMed: 19619495]

Kaufmann T., Strasser A., Jost P. J. (2011). Fas death receptor signalling: roles of Bid and XIAP. *Cell Death Differ.* 19, 42-50 [PMCID: PMC3252833] [PubMed: 21959933]

Kawai T., Takahashi K., Sato S., Coban C., Kumar H., Kato H., Ishii K. J., Takeuchi O., Akira S. (2005). IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nat. Immunol.* 6, 981-988 [PubMed: 16127453]

Kim J., Kundu M., Viollet B., Guan K. L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* 13, 132-141 [PMCID: PMC3987946] [PubMed: 21258367]

Kitada T., Asakawa S., Hattori N., Matsumine H., Yamamura Y., Minoshima S., Yokochi M., Mizuno Y., Shimizu N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* 392, 605-608 [PubMed: 9560156]

Lartigue L., Kushnareva Y., Seong Y., Lin H., Faustin B., Newmeyer D. D. (2009). Caspase-independent mitochondrial cell death results from loss of respiration, not cytotoxic protein release. *Mol. Biol. Cell* 20, 4871-4884 [PMCID: PMC2785731] [PubMed: 19793916]

Li H., Zhu H., Xu C. J., Yuan J. (1998). Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* 94, 491-501 [PubMed: 9727492]

Li Z., Jo J., Jia J. M., Lo S. C., Whitcomb D. J., Jiao S., Cho K., Sheng M. (2010). Caspase-3 activation via mitochondria is required for long-term depression and AMPA receptor internalization. *Cell* 141, 859-871 [PMCID: PMC2909748] [PubMed: 20510932]

Liang X. H., Kleeman L. K., Jiang H. H., Gordon G., Goldman J. E., Berry G., Herman B., Levine B. (1998). Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. *J. Virol.* 72, 8586-8596 [PMCID: PMC110269] [PubMed: 9765397]

Liu X. Y., Wei B., Shi H. X., Shan Y. F., Wang C. (2010). Tom70 mediates activation of interferon regulatory factor 3 on mitochondria. *Cell Res.* 20, 994-1011 [PubMed: 20628368]

Luo X., Budihardjo I., Zou H., Slaughter C., Wang X. (1998). Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell* 94, 481-490 [PubMed: 9727491]

Matsuda N., Sato S., Shiba K., Okatsu K., Saisho K., Gautier C. A., Sou Y. S., Saiki S., Kawajiri S., Sato F., et al. (2010). PINK1 stabilized by mitochondrial depolarization recruits Parkin to damaged mitochondria and activates latent Parkin for mitophagy. *J. Cell Biol.* 189, 211-221 [PMCID: PMC2856912] [PubMed: 20404107]

Medzhitov R. (2007). Recognition of microorganisms and activation of the immune response. *Nature* 449, 819-826 [PubMed: 17943118]

Meylan E., Curran J., Hofmann K., Moradpour D., Binder M., Bartenschlager R., Tschopp J. (2005). Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature* 437, 1167-1172 [PubMed: 16177806]

Mizushima N. (2010). The role of the Atg1/ULK1 complex in autophagy regulation. *Curr. Opin. Cell Biol.* 22, 132-139 [PubMed: 20056399]

Mizushima N., Levine B., Cuervo A. M., Klionsky D. J. (2008). Autophagy fights disease through cellular self-digestion. *Nature* 451, 1069-1075 [PMCID: PMC2670399] [PubMed: 18305538]

Moore C. B., Bergstralh D. T., Duncan J. A., Lei Y., Morrison T. E., Zimmermann A. G., Accavitti-Loper M. A., Madden V. J., Sun L., Ye Z., et al. (2008). NLRX1 is a regulator of mitochondrial antiviral immunity. *Nature* 451, 573-577 [PubMed: 18200010]

Murray T. V., McMahon J. M., Howley B. A., Stanley A., Ritter T., Mohr A., Zwacka R., Fearnhead H. O. (2008). A non-apoptotic role for caspase-9 in muscle differentiation. *J. Cell Sci.* 121, 3786-3793 [PubMed: 18957517]

Nakagawa T., Shimizu S., Watanabe T., Yamaguchi O., Otsu K., Yamagata H., Inohara H., Kubo T., Tsujimoto Y. (2005). Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature* 434, 652-658 [PubMed: 15800626]

Nakahira K., Haspel J. A., Rathinam V. A., Lee S. J., Dolinay T., Lam H. C., Englert J. A., Rabinovitch M., Cernadas M., Kim H. P., et al. (2011). Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. *Nat. Immunol.* 12, 222-230 [PMCID: PMC3079381] [PubMed: 21151103]

Narendra D., Tanaka A., Suen D. F., Youle R. J. (2008). Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. *J. Cell Biol.* 183, 795-803 [PMCID: PMC2592826] [PubMed: 19029340]

Narendra D. P., Jin S. M., Tanaka A., Suen D. F., Gautier C. A., Shen J., Cookson M. R., Youle R. J. (2010). PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol.* 8, e1000298 [PMCID: PMC2811155] [PubMed: 20126261]

Novak I., Kirkin V., McEwan D. G., Zhang J., Wild P., Rozenknop A., Rogov V., Lohr F., Popovic D., Occhipinti A., et al. (2010). Nix is a selective autophagy receptor for mitochondrial clearance. *EMBO Rep.* 11, 45-51 [PMCID: PMC2816619] [PubMed: 20010802]

Oberst A., Bender C., Green D. R. (2008). Living with death: the evolution of the mitochondrial pathway of apoptosis in animals. *Cell Death Differ.* 15, 1139-1146 [PMCID: PMC2612587] [PubMed: 18451868]

Oberst A., Dillon C. P., Weinlich R., McCormick L. L., Fitzgerald P., Pop C., Hakem R., Salvesen G. S., Green D. R. (2011). Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. *Nature* 471, 363-367 [PMCID: PMC3077893] [PubMed: 21368763]

Okamoto K., Kondo-Okamoto N., Ohsumi Y. (2009). Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. *Dev. Cell* 17, 87-97 [PubMed: 19619494]

Onoguchi K., Onomoto K., Takamatsu S., Jogi M., Takemura A., Morimoto S., Julkunen I., Namiki H., Yoneyama M., Fujita T. (2010). Virus-infection or 5′ppp-RNA activates antiviral signal through redistribution of IPS-1 mediated by MFN1. *PLoS Pathog.* 6, e1001012 [PMCID: PMC2908619] [PubMed: 20661427]

Pattingre S., Tassa A., Qu X., Garuti R., Liang X. H., Mizushima N., Packer M., Schneider M. D., Levine B. (2005). Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* 122, 927-939 [PubMed: 16179260]

Rabiet M. J., Huet E., Boulay F. (2007). The N-formyl peptide receptors and the anaphylatoxin C5a receptors: an overview. *Biochimie* 89, 1089-1106 [PMCID: PMC7115771] [PubMed: 17428601]

Radoshevich L., Murrow L., Chen N., Fernandez E., Roy S., Fung C., Debnath J. (2010). ATG12 conjugation to ATG3 regulates mitochondrial homeostasis and cell death. *Cell* 142, 590-600 [PMCID: PMC2925044] [PubMed: 20723759]

Ravikumar B., Moreau K., Jahreiss L., Puri C., Rubinsztein D. C. (2010). Plasma membrane contributes to the formation of pre-autophagosomal structures. *Nat. Cell Biol.* 12, 747-757 [PMCID: PMC2923063] [PubMed: 20639872]

Saitoh T., Fujita N., Jang M. H., Uematsu S., Yang B. G., Satoh T., Omori H., Noda T., Yamamoto N., Komatsu M., et al. (2008). Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. *Nature* 456, 264-268 [PubMed: 18849965]

Sandoval H., Thiagarajan P., Dasgupta S. K., Schumacher A., Prchal J. T., Chen M., Wang J. (2008). Essential role for Nix in autophagic maturation of erythroid cells. *Nature* 454, 232-235 [PMCID: PMC2570948] [PubMed: 18454133]

Scaffidi C., Fulda S., Srinivasan A., Friesen C., Li F., Tomaselli K. J., Debatin K. M., Krammer P. H., Peter M. E. (1998). Two CD95 (APO-1/Fas) signaling pathways. *EMBO J.* 17, 1675-1687 [PMCID: PMC1170515] [PubMed: 9501089]

Schroder K., Tschopp J. (2010). The inflammasomes. *Cell* 140, 821-832 [PubMed: 20303873]

Schug Z. T., Gonzalvez F., Houtkooper R. H., Vaz F. M., Gottlieb E. (2011). BID is cleaved by caspase-8 within a native complex on the mitochondrial membrane. *Cell Death Differ.* 18, 538-548 [PMCID: PMC3132005] [PubMed: 21072056]

Schulze-Osthoff K., Bakker A. C., Vanhaesebroeck B., Beyaert R., Jacob W. A., Fiers W. (1992). Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. Evidence for the involvement of mitochondrial radical generation. *J. Biol. Chem.* 267, 5317-5323 [PubMed: 1312087]

Schweers R. L., Zhang J., Randall M. S., Loyd M. R., Li W., Dorsey F. C., Kundu M., Opferman J. T., Cleveland J. L., Miller J. L., et al. (2007). NIX is required for programmed mitochondrial clearance during reticulocyte maturation. *Proc. Natl. Acad.*

Sci. USA 104, 19500-19505 [PMCID: PMC2148318] [PubMed: 18048346]

Seth R. B., Sun L., Ea C. K., Chen Z. J. (2005). Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. *Cell* 122, 669-682 [PubMed: 16125763]

Tait S. W., Green D. R. (2008). Caspase-independent cell death: leaving the set without the final cut. *Oncogene* 27, 6452-6461 [PMCID: PMC2635930] [PubMed: 18955972]

Tait S. W., Green D. R. (2010). Mitochondria and cell death: outer membrane permeabilization and beyond. *Nat. Rev. Mol. Cell Biol.* 11, 621-632 [PubMed: 20683470]

Tait S. W., Parsons M. J., Llambi F., Bouchier-Hayes L., Connell S., Munoz-Pinedo C., Green D. R. (2010). Resistance to caspase-independent cell death requires persistence of intact mitochondria. *Dev. Cell* 18, 802-813 [PMCID: PMC3004027] [PubMed: 20493813]

Tan F. J., Fire A. Z., Hill R. B. (2007). Regulation of apoptosis by C. elegans CED-9 in the absence of the C-terminal transmembrane domain. *Cell Death Differ.* 14, 1925-1935 [PMCID: PMC3047747] [PubMed: 17703231]

Tanaka A., Cleland M. M., Xu S., Narendra D. P., Suen D. F., Karbowski M., Youle R. J. (2010). Proteasome and p97 mediate mitophagy and degradation of mitofusins induced by Parkin. *J. Cell Biol.* 191, 1367-1380 [PMCID: PMC3010068] [PubMed: 21173115]

Tattoli I., Carneiro L. A., Jehanno M., Magalhaes J. G., Shu Y., Philpott D. J., Arnoult D., Girardin S. E. (2008). NLRX1 is a mitochondrial NOD-like receptor that amplifies NF-kappaB and JNK pathways by inducing reactive oxygen species production. *EMBO Rep.* 9, 293-300 [PMCID: PMC2267388] [PubMed: 18219313]

Taylor R. C., Cullen S. P., Martin S. J. (2008). Apoptosis: controlled demolition at the cellular level. *Nat. Rev. Mol. Cell Biol.* 9, 231-241 [PubMed: 18073771]

Temkin V., Huang Q., Liu H., Osada H., Pope R. M. (2006). Inhibition of ADP/ATP exchange in receptor-interacting protein-mediated necrosis. *Mol. Cell. Biol.* 26, 2215-2225 [PMCID: PMC1430284] [PubMed: 16507998]

Tooze S. A., Yoshimori T. (2010). The origin of the autophagosomal membrane. *Nat. Cell Biol.* 12, 831-835 [PubMed: 20811355]

Upton J. W., Kaiser W. J., Mocarski E. S. (2010). Virus inhibition of RIP3-dependent necrosis. *Cell Host Microbe* 7, 302-313 [PMCID: PMC4279434] [PubMed: 20413098]

Vandenabeele P., Galluzzi L., Vanden Berghe T., Kroemer G. (2010). Molecular mechanisms of necroptosis: an ordered cellular explosion. *Nat. Rev. Mol. Cell Biol.* 11, 700-714 [PubMed: 20823910]

Vives-Bauza C., Zhou C., Huang Y., Cui M., de Vries R. L., Kim J., May J., Tocilescu M. A., Liu W., Ko H. S., et al. (2010). PINK1-dependent recruitment of Parkin to mitochondria in mitophagy. *Proc. Natl. Acad. Sci. USA* 107, 378-383 [PMCID: PMC2806779] [PubMed: 19966284]

Wang K., Klionsky D. J. (2011). Mitochondria removal by autophagy. *Autophagy* 7, 297-300 [PMCID: PMC3359476] [PubMed: 21252623]

Wei M. C., Zong W. X., Cheng E. H., Lindsten T., Panoutsakopoulou V., Ross A. J., Roth K. A., MacGregor G. R., Thompson C. B., Korsmeyer S. J. (2001). Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science* 292, 727-730 [PMCID: PMC3049805] [PubMed: 11326099]

Wei Y., Pattingre S., Sinha S., Bassik M., Levine B. (2008). JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. *Mol. Cell* 30, 678-688 [PMCID: PMC2478643] [PubMed: 18570871]

West A. P., Brodsky I. E., Rahner C., Woo D. K., Erdjument-Bromage H., Tempst P., Walsh M. C., Choi Y., Shadel G. S., Ghosh S. (2011a). TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature* 472, 476-480 [PMCID: PMC3460538] [PubMed: 21525932]

West A. P., Shadel G. S., Ghosh S. (2011b). Mitochondria in innate immune responses. *Nat. Rev. Immunol.* 11, 389-402 [PMCID: PMC4281487] [PubMed: 21597473]

Westermann B. (2010). Mitochondrial fusion and fission in cell life and death. *Nat. Rev. Mol. Cell Biol.* 11, 872-884 [PubMed: 21102612]

Xu L. G., Wang Y. Y., Han K. J., Li L. Y., Zhai Z., Shu H. B. (2005). VISA is an adapter protein required for virus-triggered IFN-beta signaling. *Mol. Cell* 19, 727-740 [PubMed: 16153868]

Yang S., Wang X., Contino G., Liesa M., Sahin E., Ying H., Bause A., Li Y., Stommel J. M., Dell'antonio G., et al. (2011). Pancreatic cancers require autophagy for tumor growth. *Genes Dev.* 25, 717-729 [PMCID: PMC3070934] [PubMed: 21406549]

Yasukawa K., Oshiumi H., Takeda M., Ishihara N., Yanagi Y., Seya T., Kawabata S., Koshiba T. (2009). Mitofusin 2 inhibits mitochondrial antiviral signaling. *Sci. Signal.* 2, ra47 [PubMed: 19690333]

Youle R. J., Narendra D. P. (2011). Mechanisms of mitophagy. *Nat. Rev. Mol. Cell Biol.* 12, 9-14 [PMCID: PMC4780047] [PubMed: 21179058]

Zhang D. W., Shao J., Lin J., Zhang N., Lu B. J., Lin S. C., Dong M. Q., Han J. (2009a). RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. *Science* 325, 332-336 [PubMed: 19498109]

Zhang H., Zhou X., McQuade T., Li J., Chan F. K., Zhang J. (2011). Functional complementation between FADD and RIP1 in embryos and lymphocytes. *Nature* 471, 373-376 [PMCID: PMC3072026] [PubMed: 21368761]

Zhang J., Randall M. S., Loyd M. R., Dorsey F. C., Kundu M., Cleveland J. L., Ney P. A. (2009b). Mitochondrial clearance is regulated by Atg7-dependent and - independent mechanisms during reticulocyte maturation. *Blood* 114, 157-164 [PMCID: PMC2710944] [PubMed: 19417210]

Zhang Q., Raoof M., Chen Y., Sumi Y., Sursal T., Junger W., Brohi K., Itagaki K., Hauser C. J. (2010). Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* 464, 104-107 [PMCID: PMC2843437] [PubMed: 20203610]

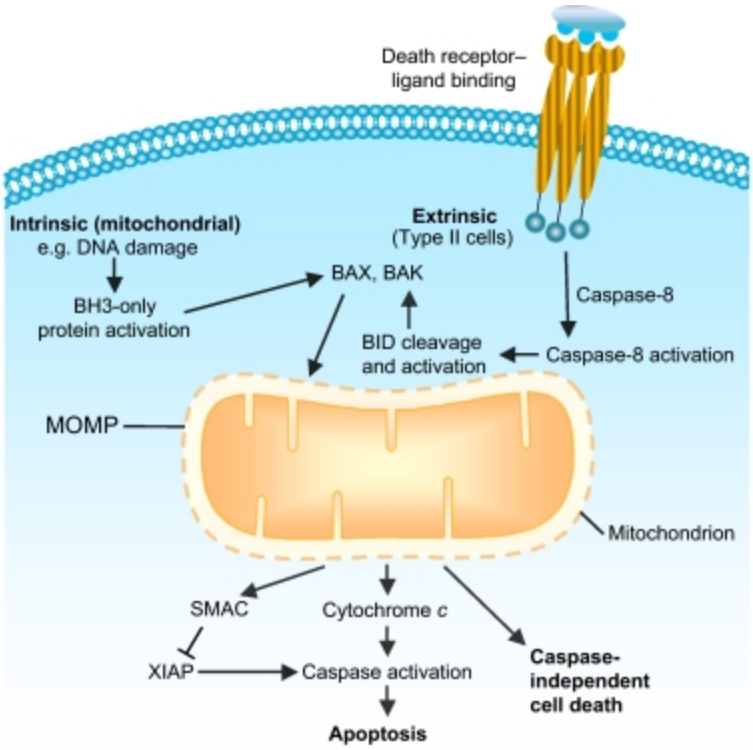
Zhong B., Yang Y., Li S., Wang Y. Y., Li Y., Diao F., Lei C., He X., Zhang L., Tien P., et al. (2008). The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. *Immunity* 29, 538-550 [PubMed: 18818105]

Zhou R., Yazdi A. S., Menu P., Tschopp J. (2011). A role for mitochondria in NLRP3 inflammasome activation. *Nature* 469, 221-225 [PubMed: 21124315]

Zoncu R., Efeyan A., Sabatini D. M. (2011). mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat. Rev. Mol. Cell Biol.* 12, 21-35 [PMCID: PMC3390257] [PubMed: 21157483]

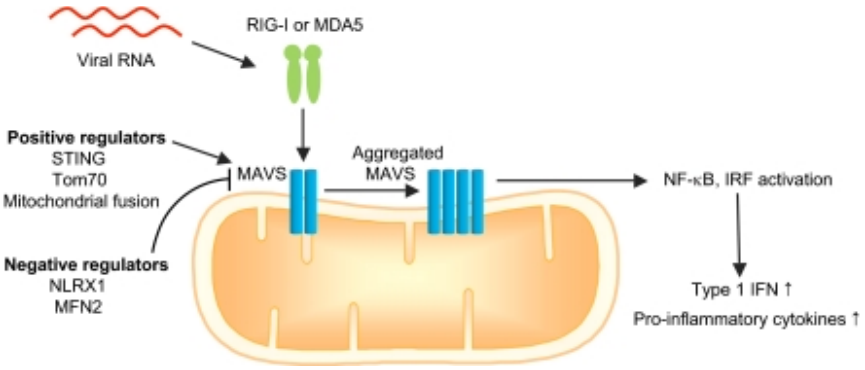
Figures and Tables

Fig. 1.



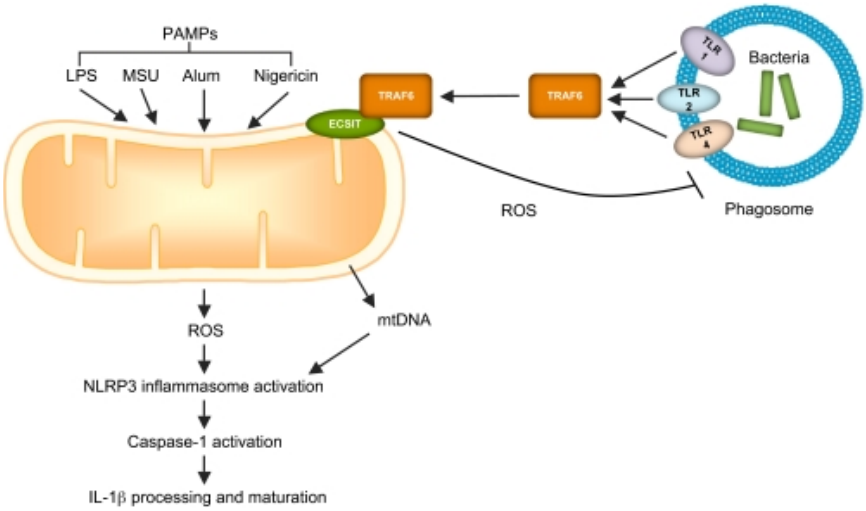
Mitochondrial regulation of apoptosis. Left: in the intrinsic pathway of apoptosis, pro-apoptotic stimuli such as DNA damage activate BH3-only proteins that, in turn, activate BAX and BAK. Active BAX and BAK cause mitochondrial outer membrane permeabilisation (MOMP), leading to release of proteins from the intermembrane space, including cytochrome *c* and SMAC (and Omi; not shown), which activate caspases and thereby cause apoptosis. MOMP can also lead to caspase-independent cell death. Right: in the extrinsic pathway, activation of cell surface death receptors leads to activation of the initiator caspase-8. In type II cells, mitochondria are required for apoptosis. Following death-receptor ligation, caspase-8 is activated at the OMM where it cleaves and activates the BH3-only protein BID, leading to BAX and BAK activation, leading to MOMP.

Fig. 2.



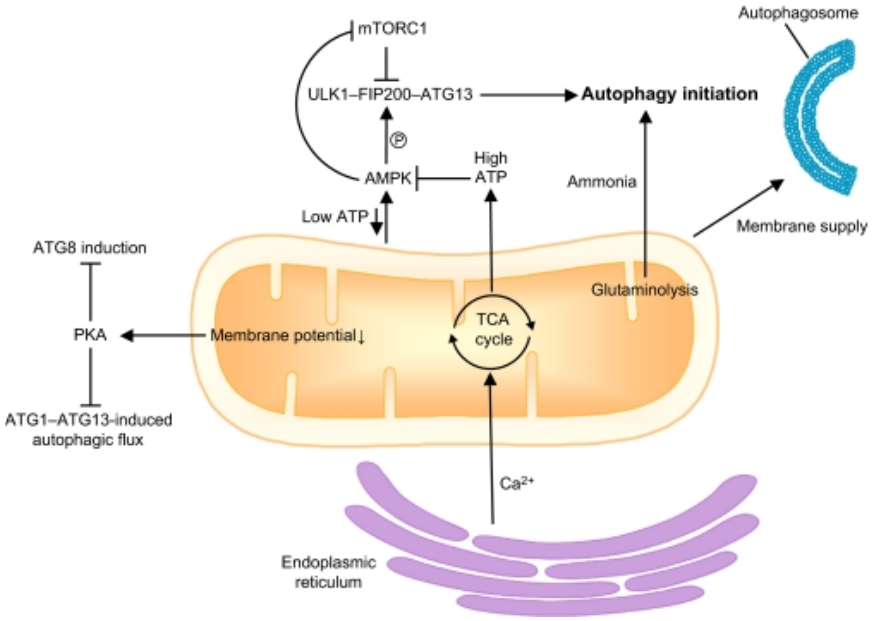
Mitochondria and antiviral signalling. Members of the RIG-I-like receptor (RLR) family, including RIG-I and MDA5, detect viral RNA, which causes their translocation to the OMM where they activate an adaptor protein called MAVS. MAVS undergoes oligomerisation, ultimately leading to upregulation of type I IFNs and pro-inflammatory cytokines. Positive regulators of MAVS activation include TOM70, STING and mitochondrial fusion, whereas negative regulators include NLRX1 and MFN2.

Fig. 3.



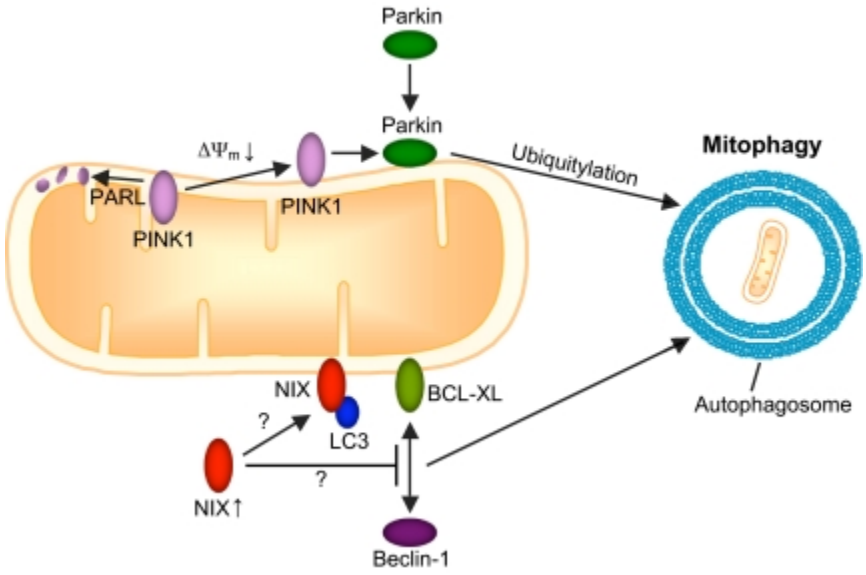
Mitochondrial ROS and innate immunity. Left: various PAMPs converge on the mitochondria, leading to upregulation of ROS. Mitochondrial ROS drive NLRP3 inflammasome activation, leading to caspase-1 activation that, in turn, cleaves and activates the pro-inflammatory cytokine IL-1β. Right: following activation, the Toll-like receptors TLR1, TLR2 and TLR4 activate TRAF6. TRAF6 translocates to the OMM and ubiquitinates ECSIT, leading to upregulation of mitochondrial ROS that have anti-microbial activity. MSU, monosodium urate, mtDNA, mitochondrial DNA.

Fig. 4.



Mitochondrial regulation of autophagy. Left: in yeast, loss of mitochondrial membrane potential leads to activation of PKA. PKA inhibits autophagy by repressing induction of ATG8 and by inhibiting ATG1–ATG13-induced autophagic flux. Centre: by regulating cellular ATP levels, mitochondria regulate autophagy through AMPK. When ATP levels are low, AMPK is activated and induces autophagy through direct phosphorylation of the ULK1–FIP200–ATG13 complex, and indirectly by inhibiting the suppression of autophagy by mTORC1. Ca^{2+} release by the ER enhances the tricarboxylic acid cycle (TCA cycle), upregulating ATP levels. Right: ammonia produced by mitochondrial-dependent glutaminolysis initiates autophagy by a non-conventional ULK1-independent mechanism. Under some conditions, the OMM can serve as a source of membrane for autophagosome biogenesis.

Fig. 5.



Parkin and NIX pathways of mitophagy. Top: in healthy mitochondria, the kinase PINK1 is imported into the mitochondrial intermembrane space and degraded in a manner dependent upon PARL protease. Following loss of mitochondrial transmembrane potential ($\Delta\psi_m$), PINK1 fails to be imported into mitochondria and accumulates on the OMM, where it recruits Parkin. Parkin that localises to the mitochondria induces mitophagy, dependent upon its ubiquitin ligase activity. Bottom: during reticulocyte maturation, NIX is upregulated and localises to the mitochondria. At the mitochondria, NIX induces mitophagy, possibly through its ability to directly bind the autophagy protein LC3 and/or its ability to block BCL-XL inhibition of the key autophagy protein beclin-1.