Mitochondria in innate immunity

Damien Arnoult¹, Fraser Soares², Ivan Tattoli² & Stephen E. Girardin^{2*}

¹INSERM U1014, Hôpital Paul Brousse, Batiment Lavoisier, Villejuif, France, and ²Department of Laboratory Medicine and Pathobiology, Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada

Mitochondria are cellular organelles involved in host-cell metabolic processes and the control of programmed cell death. A direct link between mitochondria and innate immune signalling was first highlighted with the identification of MAVS—a crucial adaptor for RIGI-like receptor signalling—as a mitochondria-anchored protein. Recently, other innate immune molecules, such as NLRX1, TRAF6, NLRP3 and IRGM have been functionally associated with mitochondria. Furthermore, mitochondrial alarmins—such as mitochondrial DNA and formyl peptides—can be released by damaged mitochondria and trigger inflammation. Therefore, mitochondria emerge as a fundamental hub for innate immune signalling.

Keywords: mitochondria; innate immunity; MAVS; pathogen; host metabolism

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See Glossary for abbreviations used in this article.

Introduction

Innate immune responses in mammalian cells rely on the detection of conserved molecular motifs found in microbial pathogens and known as 'pathogen-associated molecular patterns (PAMPs)', which include viral nucleic acids as well as bacterial molecules such as lipopolysaccharide, lipoproteins, flagellin, peptidoglycan and CpG DNA (Medzhitov, 2007). In the past few years, it has also become clear that non-microbial danger signals—composed of host molecules often released by necrotic cells in the context of tissue damage and known as 'danger-associated molecular patterns (DAMPs)'—also trigger innate immune signalling and promote inflammation (Benko *et al*, 2008). Both PAMPs and DAMPs trigger innate immunity following detection by several families of host sensor molecules, collectively called pattern recognition receptors (PRRs). The most-studied families of mammalian PRRs include Toll-like receptors (TLRs; Akira & Takeda, 2004), Nod-like receptors (NLRs; Fritz *et al*, 2006) and RIGI-like receptors (RLRs; Takeuchi & Akira, 2009). TLRs are membrane-spanning molecules that detect extracellular or luminal PAMPs and DAMPs, whereas NLRs and RLRs sense intracellular molecular signatures. Once activated, PRRs

trigger specific host defence signalling, the most commonly studied of which are pro-inflammatory pathways that activate nuclear factor κ B (NF- κ B), in response to most TLRs, the NLR family members NOD1 and NOD2, and RLRs; caspase 1 inflammasome, activated by the NLRP subfamily of NLR proteins and RLRs; and type I interferon (Elinav *et al*, 2011; Kawai & Akira, 2011; Loo & Gale, 2011). Finally, several new PRRs have been identified recently that detect intracellular foreign DNA, and these include AIM2, IFI16 and ZBP1/DLM1 (Barber, 2011).

With regards to the mechanisms that underlie PRR activation, a key remaining question is whether MAMP and DAMP detection is a direct ligand–receptor interaction or whether it requires multiple cofactors and complex modifications of host metabolic and homeostatic functions. In support of the latter hypothesis, numerous lines of evidence have recently highlighted the importance of mitochondria and mitochondrial functions in the regulation of host innate immune signalling. Importantly, although this organelle seems to be a signalling hub for innate immunity, there is strong compelling evidence to show that not only do mitochondria provide a molecular platform for signalling, but also that innate immunity and basic mitochondrial functions are integrated. Therefore, an emerging concept is that innate immune signalling is under the functional check of basic host metabolic functions, such as oxygen consumption, ATP production and possibly biosynthetic pathways that depend on mitochondrial activity and fitness.

PAMP detection and glycolytic switch

The main source of energy in the cell is the ATP that is generated through mitochondrial oxidative phosphorylation or through glycolysis. Oxidative phosphorylation is active in the presence of oxygen, whereas glycolysis is favoured under anaerobic conditions. The switch towards glycolytic ATP has recently been implicated in the activation and maturation of murine dendritic cells (Jantsch *et al*, 2008; Krawczyk *et al*, 2010). Dendritic cells stimulated with various TLR agonists have increased lactate production, higher expression of glucose transporter 1 and decreased oxygen consumption, all of which are signals of glycolytic metabolism (Krawczyk *et al*, 2010). Importantly, LPS-induced glycolysis is dependent on the PI3K/AKT pathway and could be negatively regulated by the anti-inflammatory cytokine IL-10 or AMP-activated protein kinase, a key regulator of oxidative phosphorylation (Krawczyk *et al*, 2010). In addition, macrophages are also highly dependent on glycolysis for ATP production, which is elicited by the transcription factor HIF1 α —a key regulator of hypoxia (Cramer *et al*, 2003). Although a rapid increase

¹INSERM U1014, Hôpital Paul Brousse, Batiment Lavoisier, 14 avenue Paul Vaillant Couturier, 94807 Villejuif Cedex, France

²Department of Laboratory Medicine and Pathobiology, Medical Sciences Building, University of Toronto, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada

*Corresponding author: Tel: + 416 978 7507; Fax: +416 978 5959;

E-mail: stephen.girardin@utoronto.ca

Table 1 | Viral and bacterial effectors that target mitochondria

	Anti-apoptotic factors	Pro-apoptotic factors	References
<i>Viruses</i>			
Adenovirus	E1B-19k	—	Degenhardt <i>et al</i> , 2000
Epstein–Barr virus	BHRF1	—	Henderson <i>et al</i> , 1993
Vaccinia virus	F1L, N1L	—	Wasilenko <i>et al</i> , 2003
Cytomegalovirus	vMIA	—	Kvansakul <i>et al</i> , 2007
HIV-1	—	Vpr	Jacotot <i>et al</i> , 2000
Hepatitis B virus	—	HBx	Rahmani <i>et al</i> , 2000
<i>Bacteria</i>			
<i>Legionella pneumophila</i>	SdhA	—	Laguna <i>et al</i> , 2006
<i>Escherichia coli</i>	—	EspF, Map	Nagai <i>et al</i> , 2005
<i>Helicobacter pylori</i>	—	VacA	Kozjak-Pavlovic <i>et al</i> , 2008
<i>Staphylococcus aureus</i>	—	α -toxin	Kozjak-Pavlovic <i>et al</i> , 2008
<i>Clostridium difficile</i>	—	Toxin A and B	Kozjak-Pavlovic <i>et al</i> , 2008

in ATP through glycolysis is important for macrophage function, there is evidence to suggest that glycolysis can also be a protective mechanism against inflammatory stimuli (Garedew *et al*, 2010). LPS and IFN γ stimulation normally result in nitric-oxide-dependent mitochondrial dysfunction, leading to a collapse of the mitochondrial membrane potential ($\Delta\Psi_m$) and apoptosis (Garedew *et al*, 2010). Interestingly, LPS and IFN γ -activated macrophages are less vulnerable as a result of glycolytic ATP production, which can maintain $\Delta\Psi_m$, thereby preventing cellular apoptosis (Garedew *et al*, 2010). The regulation of cellular metabolism by switching from oxidative phosphorylation to glycolysis seems to be critical in the activation and protection of certain immune cell populations.

Mitochondrial targeting by microbial effectors

Mitochondria are known to control intrinsic apoptotic cell death, and as host-cell death is a common feature of infected cells, early reports focused on a possible impact of microbial-derived proteins on host-cell viability (Arnoult *et al*, 2009a). Indeed, several viruses have been shown to block mitochondria-dependent apoptotic cell death, including those that encode viral homologues of the anti-apoptotic protein BCL2, such as the 19kDa product of the adenovirus *E1B* gene (named E1B-19K) (Degenhardt *et al*, 2000) or the Epstein–Barr virus protein BHRF1 (Henderson *et al*, 1993). Other viral proteins, such as F1L and N1L proteins from vaccinia virus and vMIA from human cytomegalovirus, block apoptosis by interfering with the pro-apoptotic proteins BAK and BAX at the mitochondrial outer membrane (Kvansakul *et al*, 2007; Wasilenko *et al*, 2003). Another group of viruses instead seems to promote host-cell apoptosis as a pathogenic mechanism. This is the case of the viral protein R of human immunodeficiency virus 1, which interacts with the mitochondrial outer membrane protein VDAC, resulting in the opening of the mitochondrial permeability transition pore (mPTP), loss of the inner

Glossary

$\Delta\Psi_m$	mitochondrial membrane potential
AIM2	absent in melanoma 2
AKT	cellular homologue of v-AKT
ASC	apoptosis-associated speck-like protein containing a CARD
ATG	autophagy-specific gene
BAK	BCL2-antagonist/killer
BAX	BCL2-associated X protein
BCL2	B-cell leukaemia/lymphoma 2
ECSIT	evolutionarily conserved signalling intermediate in Toll pathway
FPR1	formyl peptide receptor 1
FPRL1	formyl peptide receptor-like 1
gC1q	complement component 1, q subcomponent binding protein
HIF1 α	hypoxia inducible factor 1, α -subunit
IFI16	interferon, γ -inducible protein 16
IFN γ	interferon γ
IKK	I κ B kinase
IL-10	interleukin 10
IRF	interferon response factor
IRGM	immunity-related GTPase family M protein
LPS	lipopolysaccharide
MAVS	mitochondria-associated viral sensor
MDA5	melanoma differentiation associated gene 5
MFN1	mitofusin 1
MyD88	myeloid differentiation primary response gene 88
NAP1	NF- κ B-activating kinase-associated protein 1
NEMO	NF- κ B essential modifier
NLRX1	NOD-like receptor family member X1
NOX2	NADPH oxidase 2
PCBP2/AIP4	poly(rC) binding protein 2 / atrophin 1 interacting protein 4
PI3K	phosphoinositide-3-kinase
PLK1	polo-like kinase 1
Poly (I:C)	polyinosine-polycytidylic acid
RIGI	retinoic acid-inducible gene I
RNF5	RING finger protein 5
ROS	reactive oxygen species
STING	stimulator of interferon genes
TANK	TRAF family member-associated NF- κ B activator
TBK1	TANK-binding kinase 1
TRAF	TNF-receptor-associated factor
TRIF	TIR-domain containing adaptor inducing IFN- β
UQCRC2	ubiquinol-cytochrome c reductase core protein II
VDAC	voltage-dependent anion channel
ZBP1/DLM1	Z-DNA binding protein 1

membrane potential $\Delta\Psi_m$ and cytosolic leakage of host apoptogenic factors (Jacotot *et al*, 2000). The hepatitis B virus protein HBx induces similar mitochondrial collapse after interacting with VDAC3, leading to massive mitochondrial fragmentation and aggregation, and rapid apoptotic cell death (Rahmani *et al*, 2000; Table 1).

Several bacterial effectors also target mitochondria to manipulate host-cell death and/or survival pathways. Enteropathogenic *Escherichia coli* proteins EspF and Map have amino-terminal mitochondrial addressing sequences, and targeting of this organelle induces $\Delta\Psi_m$ loss and cell death (Nagai *et al*, 2005); the specific host mitochondrial proteins that interact with EspF and Map remain unknown. In addition, several bacterial toxins target mitochondria and induce cell death. This is the case of VacA from *Helicobacter pylori*, *Staphylococcus aureus* α -toxin and *Clostridium difficile* toxin A

Fig 1 | Overview of the RIGI-like receptor pathway. Viral RNAs are recognized in the cytosol by the helicases RIGI or MDA5. The amino-termini of RIGI and MDA5 contain two CARD domains that interact with the CARD domain of the mitochondrial adaptor MAVS. MAVS, after recruitment of transactivators, induces phosphorylation of IRF3 and IRF7 and NF-κB activation, leading to the production of type I IFNs and pro-inflammatory cytokines, respectively. dsRNA, double-stranded RNA; ER, endoplasmic reticulum; IκBα, inhibitor of κ light polypeptide gene enhancer in B cells, α; IKKε, IκB kinase; IRF, interferon response factor; MDA5, melanoma differentiation associated gene 5; NAP, NF-κB-activating kinase-associated protein; NEMO, NF-κB essential modifier; NF-κB, nuclear factor κB; pppssRNA, 5' triphosphate single stranded RNA; RIGI, retinoic acid-inducible gene I; STING, stimulator of interferon genes; TAB, TAK1-associated binding protein; TAK, TGF-β-activated kinase; TANK, TRAF family member-associated NF-κB activator; TBK, TANK-binding kinase; TRAF, TNF-receptor-associated factor.

and B (Kozjak-Pavlovic *et al*, 2008). *Shigella flexneri* also induces cell death in non-myeloid cells through a mechanism that depends on the opening of the mPTP pore, resulting in $\Delta\Psi_m$ loss and necrotic cell death, but whether this process depends on the injection of a specific bacterial type III secretion system effector remains unknown (Carneiro *et al*, 2009). Finally, *Legionella pneumophila* effector protein SdhA promotes survival of the infected cells by counteracting necrotic cell death as a result of mPTP opening (Laguna *et al*, 2006; Table 1).

Mitochondrial dynamics and antiviral immunity

On infection, viruses are rapidly detected by the innate immune system through several classes of PRR, including TLRs and RLRs, which recognize viral components and directly activate immune cells. On activation, TLRs recruit adaptor proteins such as MyD88 and TRIF, leading to downstream signalling cascades and production of proinflammatory cytokines such as type I interferons (IFNs) and chemokines (Akira & Takeda, 2004). Viral RNA that is synthesized in the cytoplasm of the cell or that is present in viral genomes already released into the cells is not accessible to TLR3, TLR7 or TLR8, as these

TLRs detect viral nucleic acids presented on the extracellular or luminal side of host membranes, such as endosomal compartments (Akira & Takeda, 2004). Furthermore, fibroblasts and dendritic cells that lack MyD88 and TRIF can produce type I IFN after infection with a number of viruses, suggesting that the TLR system is not always crucial for innate immune defences against viral infection (Kato *et al*, 2005).

A new pathway of TLR-independent responses to viruses was uncovered with the discovery of RIGI, the founding member of the RLR family (Takeuchi & Akira, 2010; Yoneyama *et al*, 2004), which also includes MDA5. These are cytosolic helicases that contain two CARD domains (Takeuchi & Akira, 2010; Yoneyama & Fujita, 2008) and sense viral RNA (Yoneyama & Fujita, 2008). Both helicases acquire ATPase activity by binding to their ligands, which is required to induce the conformational changes that lead to the exposure of the CARDS that are otherwise masked by the carboxy-terminal regulatory domain. This conformational change is required for a putative interaction with the CARD domain of the mitochondrial adaptor MAVS (also known as IPS1, Cardif or VISA; Kawai *et al*, 2005; Meylan *et al*, 2005; Seth *et al*, 2005; Xu *et al*, 2005). MAVS,

Fig 2 | Mitochondrial dynamics and innate immunity. In the mitochondrial outer membrane, MAVS interacts with both MFN1 and MFN2. MFN2 is a direct MAVS inhibitor. RLR activation induces MAVS oligomerization, which recruits TRAF3 and other E3 ubiquitin ligases that catalyse Lys 63 polyubiquitination of target proteins, including TRAF3. Polyubiquitin chains recruit TANK and NEMO, which in turn bind and activate TBK1, leading to the phosphorylation of IRF3. Simultaneously, MFN1 leads to the redistribution of MAVS along mitochondria and a fusion of the mitochondrial network that promotes the interaction between MAVS and STING. Low $\Delta\Psi_m$ or decreased ROS inhibit MAVS-mediated signalling. $\Delta\Psi_m$, mitochondrial membrane potential; ER, endoplasmic reticulum; IRE, interferon response factor; MAVS, mitochondria-associated viral sensor; MFN, mitofusin; NEMO, NF- κ B essential modifier; RLR, RIG-I-like receptor; ROS, reactive oxygen species; STING, stimulator of interferon genes; TANK, TRAF family member-associated NF- κ B activator; TBK, TAK1-associated binding protein; TRAF, TNF-receptor-associated factor.

through the recruitment of TRAF3 and TRAF6, then activates two cytosolic protein kinase complexes: the 'non-canonical' IKK-related kinase TBK1 or IKK-i/e associated with various adaptor proteins, such as TANK, NAP1 and NEMO; or one comprising IKK α , IKK β and NEMO (Takeuchi & Akira, 2010). The TBK1 complex induces the phosphorylation and dimerization of the transcription factors IRF3 and IRF7, which translocate to the nucleus and bind to IFN-stimulated response elements (ISREs), thereby resulting in the expression of type I IFN genes and a set of IFN-inducible genes. The IKK complex activates NF- κ B, subsequently promoting the expression of pro-inflammatory cytokines (Takeuchi & Akira, 2010; Fig 1).

The failure of MAVS-deficient mice to mount a proper IFN response to Poly(I:C) stimulation and their severely compromised immune defence against infections with RNA viruses are evidence for the essential role of MAVS in antiviral innate immunity (Kumar *et al*, 2006; Sun *et al*, 2006). Nevertheless, how MAVS-mediated signalling is engaged is not well understood. A recent study unveiled a crucial role of ubiquitin chains in IRF3 activation downstream from MAVS

through the ubiquitin-binding domains of NEMO (Zeng *et al*, 2009). In this model, NEMO is a sensor of Lys 63 polyubiquitin chains that activate TBK1 (Fig 2). However, the E3 ubiquitin ligase or ligases that mediate IRF3 activation by MAVS remain to be identified. A candidate is TRAF3, because it has been shown to be important for type I IFN production by RLRs (Oganesyan *et al*, 2006; Saha *et al*, 2006). However, in some models, the knockdown of TRAF3 does not impair the activation of the IFN β promoter (Zeng *et al*, 2009), suggesting that other E3 ubiquitin ligases can compensate for the loss of TRAF3.

Importantly, MAVS must be localized to mitochondria to exert its function (Seth *et al*, 2005), suggesting that the mitochondrial environment is required for signal transduction. In line with this, two independent studies showed that MFN1—an effector of the mitochondrial fusion machinery (Chan, 2006)—positively regulates MAVS-mediated innate antiviral responses (Castanier *et al*, 2010; Onoguchi *et al*, 2010). In both cases, MAVS was shown to interact with MFN1: after RLR activation, the complex MFN1–MAVS regulates either MAVS redistribution along mitochondria (Onoguchi *et al*,

Fig 3 | Innate immune signalling at the mitochondria. On induction of mitochondrial reactive oxygen species (ROS), NLRP3 localizes to the mitochondrial-associated endoplasmic reticulum membrane (MAM) with ASC and pro-caspase 1, inducing caspase-1 activation and the production of IL-1 β and IL-18. MAVS is located on the outer mitochondrial membrane and mediates antiviral signalling. On TLR1/2/4 stimulation, TRAF6 translocates to the mitochondria and interacts with ECSIT, contributing to ROS production. NLRX1 is internalized into the mitochondrial matrix where it interacts with UQCRC2, a protein of the complex III of the electron transfer chain, potentiating ROS production. IRGM binds to the mitochondrial lipid cardiolipin, influencing autophagy, mitochondrial fission, mitochondrial $\Delta\Psi_m$ and cell death. ASC, apoptosis-associated speck-like protein containing a CARD; ECSIT, evolutionarily conserved signalling intermediate in Toll pathway; IL, interleukin; IRGM, immunity-related GTPase family M protein; LRR, leucine-rich repeat; MAVS, mitochondria-associated viral sensor; NAD, nicotinamide adenine dinucleotide; NLRP, NOD-like receptor family, pyrin domain containing; NLRX, NOD-like receptor family member X; PYD, pyrin domain; TLR, Toll-like receptor; TRAF, TNF-receptor-associated factor.

2010) or the elongation of the mitochondrial network (Castanier *et al*, 2010). Although the role of MAVS redistribution remains to be defined, it has been proposed that the elongation of the mitochondrial network linked to RLR activation enhances the association of MAVS with STING (Castanier *et al*, 2010)—an antiviral signalling adaptor localized in the endoplasmic reticulum membrane (Ishikawa & Barber, 2008)—therefore amplifying the antiviral response (Fig 1). Surprisingly, although MFN1 seems to be a positive regulator in the RLR pathway (Castanier *et al*, 2010; Onoguchi *et al*, 2010), its close homologue MFN2 is a direct inhibitor of MAVS, a function that is probably unrelated to its role in mitochondrial dynamics (Yasukawa *et al*, 2009). Thus, although MFN1 and MFN2 have a similar function in mitochondrial fusion (Chan, 2006), it seems that they have opposite roles in viral innate immunity. As MFN1 and MFN2 both engage in homotypic and heterotypic interactions on the mitochondrial outer membrane, associations with their respective partners

might ensure fine-tuning of MAVS-mediated signalling, in addition to the regulation of mitochondrial fusion.

MAVS-mediated antiviral signalling is not only regulated by mitochondrial dynamics. Two mitochondrial proteins, NLRX1 (Moore *et al*, 2008) and gC1qR (Xu *et al*, 2009), have been recently identified as regulators of MAVS function that are involved in the inhibition of the RIGI- and MDA5-dependent antiviral response. The autophagy-related protein ATG5/12 (Jounai *et al*, 2007) and several other cellular proteins such as PCBP2/AIP4 (You *et al*, 2009), RNF5 (Zhong *et al*, 2010) and PLK1 (Vitour *et al*, 2009) have also been implicated in the negative regulation of MAVS-mediated signalling. Interestingly, a recent study has reported that $\Delta\Psi_m$ is crucial for this pathway (Koshiba *et al*, 2011). Cells in which the $\Delta\Psi_m$ was dissipated—treated with the protonophore CCCP or lacking both MFN1 and MFN2—are defective in the antiviral innate immune response after RLR, but not TLR3, activation. The precise role of $\Delta\Psi_m$ in

MAVS-mediated signalling remains to be determined, although Koshiba and colleagues suggest that loss of $\Delta\Psi_m$ might prevent the structural rearrangement of the MAVS complex, which could exist as a readily available pool on the mitochondrial membrane to provide a quicker response to viral infection (Koshiba *et al*, 2011). Furthermore, loss of $\Delta\Psi_m$ is associated with an intensive fragmentation of the mitochondrial network that might prevent the association of MAVS with the endoplasmic-reticulum-associated antiviral signalling adaptor STING (Castanier *et al*, 2010; Ishikawa & Barber, 2008). Importantly, another conclusion of this study is that the inhibition of ATP synthesis—one of the most prominent functional role of mitochondria—does not impair MAVS-mediated signalling.

An indirect role of mitochondria in the regulation of innate immune responses is shown by the fact that ROS modulate several signalling pathways, including the NF- κ B and JNK pathways, and the caspase 1 inflammasome (Naik & Dixit, 2011). The two main sources of cellular ROS are the mitochondria and the membrane-associated NADPH oxidase (NOX) and dual oxidase (DUOX) complexes. NOX2 and ROS were recently shown to be required for the triggering of efficient MAVS-mediated antiviral signalling (Soucy-Faulkner *et al*, 2010). The authors also provide evidence that NOX2 is crucial for the expression of MAVS because NOX2 down-regulation diminishes MAVS mRNA expression without affecting its mitochondrial localization (Soucy-Faulkner *et al*, 2010).

Although the function of mitochondria in MAVS-mediated antiviral signalling warrants further investigation because mitochondria are essential for MAVS function (Seth *et al*, 2005), recent studies have suggested that mitochondrial dynamics, $\Delta\Psi_m$ and ROS are crucial regulators and/or effectors of this pathway (Fig 2).

Mitochondria: central platform for innate immunity

The identification of MAVS as a central adaptor protein of the RLR pathway that localizes to the mitochondrial outer membrane provided the first example of a functional link between mitochondrial function and innate immune signalling. Notably, recent evidence demonstrates that many host defence pathways are controlled by this organelle (Fig 3).

NLRP3. NLRP3 is a crucial NLR protein implicated in the induction of the caspase 1 inflammasome in response to a surprisingly wide spectrum of microbial-associated and danger-associated molecular signatures (see Benko *et al* (2008) for a recent comprehensive review on this topic). The pleiotropic nature of the upstream activators of NLRP3 has led to speculation that NLRP3 senses a common cellular signal triggered by PAMPs and DAMPs rather than several individual molecular entities. The nature of this common signal is still open to debate, as evidence suggests that either lysosomal damage or ROS could act as NLRP3 activators (Tschopp & Schroder, 2010). Although phagosome-associated ROS generated by the NADPH complex were first proposed to trigger NLRP3 responses, these observations have been challenged by the fact that normal NLRP3 responses occur in cells carrying mutations that impair NADPH-oxidase-dependent ROS production (van Bruggen *et al*, 2010). The group of Jürg Tschopp recently proposed that mitochondria could be the main source of ROS contributing to NLRP3 activation (Zhou *et al*, 2011), providing a possible explanation for these conflicting results. They found that rotenone and antimycin—two drugs that block the mitochondrial respiratory chain and lead to the generation of mitochondrial ROS—potentially activate NLRP3. Similarly,

inhibition of autophagy results in the accumulation of defective and ROS-producing mitochondria, which potentiates NLRP3-dependent inflammasome activation. Interestingly, the authors also demonstrated that mitochondria act as a molecular scaffolding platform for the recruitment of several members of the NLRP3 inflammasome—including NLRP3 itself and the adaptor protein ASC—in response to known NLRP3 activators, such as monosodium urate, aluminium hydroxide and nigericin (Zhou *et al*, 2011). The mechanism that underlies NLRP3 inflammasome recruitment to the vicinity of ROS-producing mitochondria remains unknown, and future studies are necessary to identify the mitochondria-associated docking complexes for NLRP3 inflammasome components.

TLRs. The engulfment of bacteria by phagocytic cells results in the activation of numerous innate immune signalling pathways, including those that promote microbicidal activity. ROS generation is one of the most ancient and efficient means to kill microorganisms and it is functionally linked to phagocytosis through the upregulation of the NADPH oxidase complex, which is localized at the surface of the phagosome. TLR signalling enhances NADPH oxidase activity and, in turn, ROS generated by this complex potentiates TLR-mediated induction of inflammatory pathways, such as those dependent on NF- κ B and MAP kinases (Gill *et al*, 2010). A recent study showed that mitochondria might also have crucial roles in phagosome-associated ROS generation and in the connection to TLR signalling. Indeed, activation of murine macrophages with ligands of cell-surface TLRs—TLR1, TLR2 and TLR4—but not of endosomal TLRs—TLR3, TLR7, TLR8 and TLR9—resulted in the production of mitochondria-derived ROS (West *et al*, 2011). Interestingly, when cells phagocytosed latex beads coated with TLR2 ligand Pam3CSK4 or TLR4 ligand LPS, mitochondria accumulated near to phagosomes containing the latex beads, an effect that was not observed if beads were coated with the endosome-associated TLR9 ligand CpG DNA. The authors concluded that engulfment of microbes, concomitant with TLR-mediated detection, resulted in mitochondrial accumulation around phagosomes and local production of ROS, which could contribute to the elimination of the engulfed microorganism. TRAF6—an E3 ligase crucial for TLR signalling—is translocated to the mitochondria in response to the engagement of TLR1/2/4 ligands, but not TLR3/9 ligands, and mitochondrial ECSIT protein was identified as a TRAF6-dependent target of ubiquitination after macrophage activation by TLR1/2/4 ligands. Importantly, triggering of the TRAF6–ECSIT pathway at the mitochondria was responsible for the recruitment of these organelles to the vicinity of phagosomes and the induction of mitochondrial ROS. Therefore, this study highlights the unexpected importance of mitochondrial ROS generation in phagosomal killing, and provides a new example of how mitochondria can act as a signalling hub—in this case, the TRAF6–ECSIT pathway—for innate immune signalling (West *et al*, 2011).

NLRX1. NLRX1 is a member of the NLR family that has a unique N-terminal domain—which accounts for the letter 'X' in its acronym—that was found to contain a mitochondrial addressing sequence (Moore *et al*, 2008; Tattoli *et al*, 2008). Biochemical analyses demonstrated that NLRX1 localizes to the mitochondrial matrix (Arnoult *et al*, 2009b). Strikingly, NLRX1 represents the first and so far only example of a PRR family member that targets this cellular location, therefore implying that NLRX1 probably establishes a fundamental link between mitochondrial functions and innate

Fig 4 | Release of mitochondrial DAMPs triggers cell death and inflammatory pathways. (A) Severe trauma can induce the release of mitochondrial DNA (mtDNA), which is detected by TLR9 located on the neutrophil surface, and thus activates p38 MAP kinase. N-formyl peptides are released by mitochondria and recruit polymorphonuclear neutrophils (PMNs) by binding to the formyl-peptide receptor 1 (FPR1) expressed on their surface. (B) Autophagy-deficient cells accumulate defective mitochondria, which release mtDNA into the cytosol, triggering activation of the inflammasome through an undefined cytosolic sensor. (C) Cell death signals induce the release of cytochrome c into the cytosol, leading to the recruitment of APAF1 and pro-caspase 9 into a multiprotein complex known as the apoptosome. After activation, caspase 9 induces apoptosis by cleaving effector caspases. DAMP, danger-associated molecular pattern; IL, interleukin; MAPK, mitogen-activated protein kinase; TLR, Toll-like receptor.

immunity. However, the fact that NLRX1 targets the mitochondrial matrix is puzzling, as this confined location makes it unlikely that the protein would directly detect microbial-derived molecular signatures in an archetypal manner. Thus, the exact function of NLRX1 is unclear. Overexpression of NLRX1 was shown to induce ROS production (Tattoli *et al*, 2008), which can be explained by its interaction with UQCRC2, a matrix-facing component of the mitochondrial respiratory chain complex III that has a crucial role in ROS generation (Arnoult *et al*, 2009b). In *Chlamydia*-infected cells, NLRX1 potentiates the ROS production triggered by the pathogen, an effect that is required for optimal bacterial growth (Abdul-Sater *et al*, 2010). Interestingly, NLRX1 was also found to interact with MAVS, the crucial adaptor protein of RLR pathways, on the cytosolic side of the mitochondrial outer membrane, resulting in inhibition of MAVS-dependent antiviral responses (Moore *et al*, 2008). However, how NLRX1 interacts with MAVS remains unclear because these two proteins are targeted to distinct mitochondrial subdomains that are separated by two membranes. As NLRX1 entry into mitochondria was shown to depend on the inner membrane potential $\Delta\Psi_m$ (Arnoult *et al*, 2009b), it is conceivable that dissipation of $\Delta\Psi_m$ would result

in the retention of NLRX1 in the cytosol where the protein could interact with and inhibit MAVS-dependent signalling. This hypothesis has not been tested experimentally and it would be important to determine whether viral infection could induce cytosolic retention of NLRX1. Alternatively, NLRX1 could influence RLR signalling through ROS production, which has been shown to modulate MAVS-dependent pathways (Soucy-Faulkner *et al*, 2010).

IRGM. IRGM is a ubiquitously expressed human guanosine triphosphatase (Hunn *et al*, 2011) that functions in the innate immune control of intracellular pathogens. In contrast to the many murine IRG genes that are regulated by interferons, only two orthologues exist in humans—IRGM and IRGC. IRGM was initially thought to be a pseudogene but has a role in the elimination of *M. tuberculosis* in human macrophages (Singh *et al*, 2006). Specifically, IRGM was demonstrated to be crucial in rapamycin-, starvation- and IFN γ -induced autophagy to restrict the intracellular growth of *M. tuberculosis* in infected human macrophages (Singh *et al*, 2006). Importantly, single nucleotide polymorphisms in IRGM have been identified as risk factors for Crohn's disease (Parkes *et al*, 2007) and susceptibility

Sidebar A | In need of answers

- (i) Why does MAVS have to be anchored to the mitochondrial outer membrane to exert its function?
- (ii) What is the precise role of $\Delta\Psi_m$ in MAVS-mediated signalling?
- (iii) Do some BCL2 family members also regulate MAVS-mediated innate immunity?
- (iv) Which signals activate NLRX1 in the mitochondrial matrix?
- (v) To what extent do mitochondrial metabolic functions have an impact on the innate immune pathways that require this organelle as a signalling hub?
- (vi) What is the role of mitochondria in innate immunity in other organisms, such as *Drosophila*?

to tuberculosis (Intemann *et al*, 2009) in different human populations. The mechanism of IRGM-mediated autophagy was largely unknown, but a recent study from the Deretic group has revealed the importance of mitochondria in this process. IRGM localizes to mitochondria and regulates mitochondrial fission events, which had been previously implicated in autophagic control of intracellular pathogens (Singh *et al*, 2010). The localization of IRGM to mitochondria is dependent on the interaction of IRGM with cardiolipin, a mitochondrial lipid found predominately in the inner mitochondrial membrane. Additionally, a detailed analysis of IRGM spliced variants indicated that preferential overexpression of IRGMd isoform induces mitochondrial fission, mitochondrial depolarization and autophagy-independent cell death, leading to the release of HMGB1, a major pro-inflammatory alarmin/DAMP (Singh *et al*, 2010). Altogether, the dynamic regulation of mitochondria seems to be a central aspect of the dual function of IRGM in mediating autophagy (cell survival) and apoptosis and/or necrosis (cell death) in innate immunity. Future studies need to explore the role of intra-mitochondrial IRGM in the context of bacterial infection.

Modulation of innate immunity by mDAMPs

Mitochondria probably evolved from a bacterial ancestor that established a symbiotic relationship with its host cell and, in this regard, mitochondrial DAMPs (herein termed 'mDAMPs') can be viewed as intermediate entities between DAMPs and PAMPs. Not surprisingly, mitochondria have several unique features in the cell and several mitochondrial elements—such as cytochrome *c*, formyl peptides and mitochondrial DNA (mtDNA)—seem to have been selected during evolution to act as mDAMPs that alert the cell to damage in the mitochondria. By far the most studied mDAMP is cytochrome *c*, which triggers apoptotic cell death after its release from the mitochondrial inner membrane into the cytosol, where it interacts with APAF1 to trigger the activation of the caspase-9-dependent pathways of apoptosis. Although mitochondria-dependent apoptotic cell death is a crucial aspect of innate immune responses to pathogens, it has been extensively reviewed elsewhere (Garrido *et al*, 2006) and is not discussed here. Instead, we focus on more recent aspects of the link between mDAMP liberation and innate immunity (Fig 4).

Circulating mDAMPs in trauma. Zhang and colleagues hypothesized that, in trauma patients and organ injuries without an open wound, cellular damage would lead to the release of mitochondrial molecules that could operate as mDAMPs and initiate an inflammatory response (Zhang *et al*, 2010a). The authors noticed a considerable increase in the concentration of mtDNA in the circulation of trauma

patients as compared with healthy volunteers. MtDNA released at the site of the injury recruited polymorphonuclear neutrophils (PMNs), which were also activated through a TLR9-dependent pathway. In addition to mtDNA, mitochondrial formyl peptides were also proposed to act as mDAMPs during injury. Stimulation of the G-protein-coupled receptors FPR1 and FPRL1—which mediate the chemotactic and stimulatory effects of N-formyl-Met-Leu-Phe (fMLP)—was shown to occur on PMNs after exposure to mDAMPs. This indirectly suggests that endogenous mitochondrial formyl peptides are liberated *in vivo* at the site of injury and in the circulation. Therefore, liberation of mDAMPs can account for the induction of sterile inflammation after organ injury and in trauma patients, which can result in a systemic inflammatory response syndrome similar to that observed during septic shock.

MtDNA and inflammasome activation. There has recently been increasing interest in the identification of intracellular sensors of foreign DNA (Barber, 2011), and type I IFN induction and inflammasome activation seem to represent two of the main host responses to intracellular DNA detection. Several PRRs have been shown to mediate the host response to intracellular DNA: ZBP1/DLM1 is crucial for the induction of type I IFN in some cell types, whereas in others the conversion of DNA into RNA by the RNA polymerase III has been shown to redirect host responses to the RIGI/MAVS pathway (Barber, 2011). However, the physiological relevance of the latter system in the context of bacterial infection has been challenged (Monroe *et al*, 2009). Recently, the PYHIN protein IFI16 (p204 in mice) was also shown to detect cytosolic DNA, leading to the induction of type I IFN after recruitment and activation of STING, which seems to act as a key adaptor molecule for several intracellular DNA-sensing systems (Unterholzner *et al*, 2011). In addition, activation of the caspase 1 inflammasome in response to foreign DNA requires AIM2, another cytosolic PYHIN protein (Barber, 2011).

MtDNA can be released from damaged mitochondria (Zhang *et al*, 2010b) and—as mtDNA has common features with bacterial genomes—the potential role of mtDNA in the activation of host responses to cytosolic DNA is starting to be analysed. The inhibition of autophagy results in the accumulation of defective mitochondria and the cytosolic release of mtDNA (Nakahira *et al*, 2011). Interestingly, the presence of cytosolic mtDNA—and not the accumulation of damaged mitochondria—was shown to induce activation of the caspase 1 inflammasome in a partly AIM2-independent manner. This suggests that an unknown cytosolic sensor of mtDNA might contribute to the activation of the inflammasome.

Concluding remarks

Accumulating evidence has introduced the concept that mitochondria—in addition to their well-known role in cell metabolic processes and cell death—are crucial assembly platforms for innate immune signalling, as summarized in this review. Among the outstanding questions that remain poorly understood (Sidebar A) is: why mitochondria? An attractive possibility is that the convergence of multiple innate immune pathways on this organelle would allow for a tight functional integration of host defence and metabolic processes, although this model has not yet been thoroughly tested. Could it be that the activation of certain innate immune responses requires transition through an 'energetic checkpoint'? This could ensure that efficient cell-intrinsic defence pathways would only be turned on in cells that remain fit. Another probable reason for the functional coupling of some innate

immune pathways to mitochondria is that this organelle is a major cellular source of ROS, which contribute in many ways to host defence against pathogens. The topological organization of host defence pathways at the mitochondria might therefore have evolved because ROS are intrinsically unstable molecules that cannot diffuse into the cell.

Mitochondria have been studied for decades regarding their role in cellular metabolism. Equally as unexpected as the discovery 20 years ago that this organelle controls programmed cell death, the mitochondrion now emerges as a key component in host innate immune defence pathways. The implications of this new paradigm are only starting to emerge.

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[From left to right] Ivan Tattoli, Stephen E. Girardin, Fraser Soares & Damien Arnould