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Mitochondrial Factors In The Regulation Of Innate Immunity

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Abstract

Viral infection stimulates multiple signalling pathways in the innate immune system, leading to type 1 interferon production. Recent research has identified the mitochondrial protein MAVS as a key component of one intracellular pathway, definitively linking mitochondria to the mammalian antiviral defence system for the first time.

Keywords

MAVS; IPS-1; Cardif; VISA; Mitochondria; Innate Immunity; CARD domain

1. Introduction

The mammalian innate immune system is the first line of defence against microbial and viral pathogens, and has evolved to provide a highly specific response to a vast number of potential invaders. This ancient system, shared by plants and animals (and therefore likely to have developed prior their evolutionary divergence [1]), relies on recognition of the molecular markers inherent in pathogens to differentiate self from non-self. Pathogen-associated molecular patterns (PAMPs), such as dsRNA, unmethylated CpG DNA and lipopolysaccharide, are recognized by receptors expressed in cells involved in the innate immune response (Fig. 1). These pattern-recognition receptors (PRRs) then act to initiate signalling cascades that culminate in a variety of anti-pathogenic outcomes, ranging from the production of cytokines, to the apoptotic clearance of an infected cell.

PRRs of the innate immune system can be placed into two categories, based on where they function and recognize specific PAMP signals. Extracellular pathogens are dealt with by members of the Toll-Like Receptor (TLR) family, a group of transmembrane proteins expressed at the cell surface or within endosomal compartments (reviewed in [2]). TLRs are highly specific receptor proteins, with each member generally responding to a different type of PAMP. Once activated, each TLR signals through a cytoplasmic toll/interleukin-1 receptor (TIR) domain to an adaptor such as TRIF or MyD88, and this initiates a signalling cascade that induces the production of interferons or inflammatory cytokines. Pathogens that make it into a host cell without encountering the TLR proteins are met with a range of intracellular PRRs, and this second group consists of members such as Protein Kinase R (PKR), the 2’-5-Oligoadenylate Synthetase (OAS)/RNaseL pathway, the NOD-Like Receptor (NLR) family and the RIG-I-like helicase (RLH) pathway [1,3]. PKR is activated by binding to the dsRNA

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produced during viral replication, and has a multiple antiviral properties, including: (1) activation of type-1 interferon signalling pathways; (2) phosphorylation and inactivation of the translation initiation factor eIF2α, which blocks viral (and host cell) protein synthesis; and (3) induction of apoptosis in infected cells to limit viral spread [1]. The 2'5'-OAS/RNaseL pathway also limits viral spread by inducing apoptosis, this time by cleaving all viral and cellular RNA. 2'-5'-OAS binds to intracellular viral RNA, stimulating the production of 2'-5'-oligoadenylate, which in turn activates the endoribonuclease RNaseL. RNaseL cleaves all small RNA molecules (both host and viral), which blocks protein synthesis and induces apoptosis [3,4]. The NLR family mainly consist of a number of cytoplasmic leucine-rich repeat (LRR) proteins with diverse functions, such as the activation of NF-κB and the production of the inflammatory cytokine IL-1β [5,6].

The most recently discovered cytoplasmic antiviral PRRs are members of the RLH pathway, including Retinoic-Inducible Gene-I (RIG-I). This caspase activation and recruitment domain (CARD) protein is a DEAD-box RNA helicase that binds to dsRNA and ssRNA of viral origin, initiating a type-1 interferon response [7,8]. While not constitutively active, overexpression of the full-length protein has the ability to potently upregulate antiviral transcription during infection, and was identified through its ability to enhance interferon regulatory factor (IRF)-activation in combination with synthetic viral dsRNA [7]. Importantly, expression of a CARD-only truncation mutant of RIG-I was able to initiate antiviral signalling without viral cofactors, showing the importance of this domain [7]. The RLH family also contains Melanoma Differentiation-Associated 5 (MDA-5), which has a similar domain structure and acts in a similar manner as RIG-I, but is activated by different viruses; and Laboratory of Genetics and Physiology 2 (LGP2), which again is similar to RIG-I in structure, but has no CARD domain and acts as a negative regulator of the other two proteins [8].

2. Identification of a mitochondrial member of the RLH pathway

Following the discovery of the RIG-I-like helicases, a great deal of research effort was expended to find interacting proteins that may be in the RLH pathway, or that may regulate RIG-I function. In 2005, four independent groups simultaneously reported a new member of the intracellular innate immune system signalling pathway, with each group giving the same protein a different name. Seth et al [9] discovered the Mitochondrial Antiviral Signalling protein (MAVS) by performing sequence similarity searches, using the CARD domain of MDA-5 as bait, and noted that the protein had been found in a previous screen of NK-κB inducers [10]. They reported that overexpression of MAVS activated IRF3 and NK-κB, and that its presence was required for an immune response to sendai virus infection [9]. Deletion studies showed that both the N-terminal CARD domain and C-terminal transmembrane domain were required for function, and that this protein operated downstream of RIG-I in the RLH pathway [9]. Kawai et al [11] found Interferon-β Promoter Stimulator 1 (IPS-1) by screening cDNA pools for novel inducers of the IFN-β promotor. They reported that IPS-1 could activate the IRF3, IRF7 and NK-κB promoters to induce interferon production, and that it interacted with RIG-I and MDA-5 through its CARD domain [11]. Meylan et al [12] found CARD Adaptor Inducing IFN-β (CARDIF) using a similar sequence similarity search as Seth et al [9], and demonstrated that it could recruit the signalling kinases IKKα, IKKβ and IKKε to its C-terminal region to stimulate the IRF3 and NK-κB transcription factors. Using an elegant *in vitro* infection system, the authors also demonstrated that CARDIF was cleaved by the NS3/4A protease of the hepatitis C virus, showing for the first time that viruses have evolved ways to specifically disrupt CARDIF signalling [12]. Finally, Xu et al [13] made expression constructs of the 12 unknown proteins from the Matsuda et al study of NK-κB activators [10], and found that Virus-triggered IFN-β Signalling (VISA) was the only one to significantly induce NK-κB signalling. Using a combination of yeast two-hybrid and mammalian co-

immunoprecipitation studies, they found that VISA interacted with both TRAF2 and TRAF6, thereby implicating it in TLR3-mediated signalling pathways.

Crucially, only Seth et al [9] in these primary papers localized this novel protein to mitochondria and for this reason, in conjunction with the recommendations of the Human Genome Organisation Nomenclature Committee (http://www.genenames.org/), this protein will be referred to as "MAVS" for the remainder of this review.

3. The structure and function of MAVS

MAVS consists of three distinct functional domains within a 540 residue protein – an N-terminal CARD domain (10 – 77 aa), a medial proline-rich region (PRR; 103 – 173 aa) and a C-terminal transmembrane domain (TM; 514 – 535 aa) (Fig. 2). The CARD domain consists of a six-helix bundle that contains two polar surfaces at opposite ends of the molecule, and which are predicted to mediate homotypic CARD:CARD protein interactions [14]. Activation of RIG-I or MDA-5 by viruses leads to their association with MAVS through this domain, and consequently CARD-minus deletion mutants have ablated signalling function [9,11,12]. While several other CARD domain proteins exist, there have been no conclusive reports to date of MAVS CARD:CARD interactions with proteins other than RIG-I or MDA-5, possibly because these proteins contain the greatest sequence similarity to the MAVS CARD domain [14]. The PRR domain is a proline-enriched section of the MAVS protein, with several consensus binding sites for proline-interacting proteins. For example, the Tumour Necrosis Factor Receptor Associated Factor (TRAF) family members TRAF2, TRAF3 and TRAF6 have been shown to bind to MAVS at their respective interaction site within the PRR [13,15]. The PRR also contains several P-x-x-P motifs that are required for binding by members of the Src Homology 3 (SH3) family of proteins [16], however no interaction partners of this type have yet been reported.

The C-terminal TM of MAVS anchors it to the mitochondrial outer membrane, and has structural similarity to other known mitochondrial membrane proteins such as Bcl-xL, Bcl-2 and TOM20 [9]. Removal of this portion changes the localization of MAVS, and this truncated protein is found uniformly distributed within the cytosol when exogenously expressed [9]. The importance of the mitochondrial localization, and hence the TM domain, was demonstrated by studies showing that its removal ablated MAVS signalling to NF-κB and IFN-β [9,13]. Further, an elegant experiment by Seth et al [9] demonstrated that replacement of the TM domain with the equivalent portion of Bcl-xL or Bcl-2 restored both mitochondrial localization and immune signalling, while directing MAVS to the plasma membrane or the endoplasmic reticulum membrane left it with only a small relative activity. Two recent papers have implicated the TM domain in MAVS homodimerization, suggesting that signalling is dependent on the ability of MAVS to self-associate [17,18]. In summary, both the CARD and TM domains of MAVS appear to be most important for its antiviral function.

In many reports examining endogenous MAVS, several immunoreactive bands appear on western blots using a MAVS antibody, in addition to one at the expected 57 kD molecular weight (see e.g. [9,19,20]). RT-PCR analysis of HeLa cell RNA has identified at least three new splice variants of MAVS, termed MAVS 1a (exon 2 deletion), MAVS 1b (exon 3 deletion) and MAVS 1c (lacking exon 6) [21]. MAVS 1c lacks the TM domain and is expected to have no signalling activity; however the other two splice variants (which have frame-shift mutations caused by the loss of exons) were found to have diverse biological functions. MAVS 1a has a partial CARD domain and shares only the first 39 amino acids with the full-length protein, while MAVS 1b shares the first 97 amino acids (including the full CARD domain), but lacks the PRR and TM domains [21]. In overexpression studies, MAVS 1a interacted biochemically with TRAF2, TRAF6 and RIP1, and potently inhibited the activation of IL-8 and IFN-β promoter constructs, indicating that it may act as an endogenous inhibitor of MAVS function

[21]. In contrast MAVS 1b bound FADD and RIP1, and selectively activated the IFN-β promoter, allowing it to promote an immune response against vesicular stomatitis virus infection [21]. It will be of great interest to know if these splice variants, particularly the endogenous inhibitor, function in the same manner *in vivo* as these overexpressed constructs.

4. Regulation of MAVS signalling by interacting proteins

Many proteins have been implicated in the signalling events that occur downstream of MAVS during infection, however there is still much discussion over their relative importance in terms of actual interferon production. Several groups have shown that members of the TRAF protein family, specifically TRAF2, TRAF3 and TRAF6 bind to MAVS in the PRR and are required for downstream activation of NF-κB and/or IRF3/7 [9,13,15]. However, there was no loss of IFN-β activation in TRAF6-deficient MEFs and, importantly, a truncated mutant of MAVS lacking all TRAF binding sites was fully capable of activating interferon production [9], which brings into question the importance of this binding. Association of MAVS with FADD and RIP1 [10, 21] may link the RLH pathway to the IKK complex of IKKα-IKKβ-IKKγ/NEMO and, hence, activation of NF-κB. However loss of RIP1 does not inhibit IFN-β activation in MEFs [9], suggesting that this pathway is not essential. Phosphorylation of IRF3 appears to be closely linked to the kinases TBK-1 and, particularly, IKKε, which interacts with MAVS at the mitochondrial outer membrane during infection [13,22]. From these data, it would appear that MAVS may be involved in several, possibly overlapping, interferon pathways, and it is likely that the importance of each will vary in a cell-type-dependent manner.

In many cases, viral persistence relies on the ability of viruses to evade the innate immune system. In terms of MAVS, several viruses have evolved ways to disrupt downstream signalling by targeting it for proteolytic cleavage. Hepatitis C virus (HCV) encodes NS3/4A, a serine protease that had previously been shown to disrupt the TLR protein TRIF by cleavage, and also to block RLH signalling by targeting an unidentified protein [23,24]. A number of contemporaneous studies showed that NS3/4A cleaves MAVS at Cys508 to remove it from the outer mitochondrial membrane, which in turn blocked MAVS-dependent interferon production and aided HCV persistence [12,22,23]. GB virus B (GBV-B), a close relative of HCV in small primates, also uses the same serine protease to cleave MAVS and block its function [25]. Hepatitis A virus (HAV), a member of the *Picornaviridae* and therefore in a different family to HCV, performs a similar cleavage-based inhibition of MAVS using the cysteine protease 3ABC. This structural protease, which cleaves at Gln428, also removes the MAVS from mitochondria to abrogate downstream activation of the IFN-β promoter [19]. These data suggest that this strategy of blocking RLH signaling in this manner has evolved multiple times, and makes MAVS an interesting target for therapeutic intervention.

Signalling through the RLH pathway has recently been linked to the regulation of autophagy, a process primarily associated with the controlled degradation of cellular components. Loss of the autophagy-related gene Atg5 in mice leads to an amplification of type 1 interferon production and increased resistance to vesicular stomatitis virus [26,27]. Immunoprecipitation studies have shown that Atg5 (and its conjugate partner Atg12) could bind directly with the CARD domains of MAVS and RIG-I, and the authors suggested that this interaction mediated the CARD:CARD association of these two proteins during infection [26]. However, it is unclear why two related proteins that have been shown to associate directly, and that come from a family where homotypic interactions are the norm, would require an unrelated protein to enhance their interaction. An alternative explanation for the importance of autophagy in antiviral signalling comes from studies which show that loss of Atg5 leads to accumulation of damaged mitochondria and, consequently, an increase in reactive oxygen species (ROS) production [27]. The increase in interferon production in $Atg5^{-/-}$ MEFs is potentially a result of either an upregulation of MAVS-mediated signalling (caused by the relative increase in

MAVS levels), the higher production of ROS (a known stimulator of NF-κB and other immune-promoting proteins [6]), or both.

5. The relationship between MAVS and apoptosis

Mitochondria are key components of the mammalian intrinsic apoptosis system, and proteins located there regulate many of the early steps involved in the induction of cell death. For example, the Bcl-2 family of proteins contain pro- and anti-apoptotic members, which act early in this cell death pathway to control how cells react to apoptotic stimuli. Pro-apoptotic members (such as Bax and Bak) react to lethal stimuli by coalescing on the outer mitochondrial membrane in large oligomers, while anti-apoptotic members (such as Bcl-2 and Bcl-xL) inhibit this redistribution (see [28] for a comprehensive review). If the pro-apoptotic members succeed, cytochrome *c* is released from the mitochondrial inner membrane space, which leads to the activation of caspases and eventual destruction of the cell. Given that MAVS is found on the outer mitochondrial membrane, and the link between apoptosis and the innate immune system [1,3,4], there was speculation that MAVS may link the two processes [29]. In their original MAVS paper, Seth et al [9] demonstrated that siRNA knockdown of MAVS led to cleavage of the apoptosis marker poly(ADP-ribose) polymerase (PARP), indicating that MAVS may have a cytoprotective effect. However, the same group later reported that in $MAVS^{-/-}$ mice there was no noticeable change in PARP cleavage and caspase-3 activation following UV irradiation [30]. Holm et al [31] demonstrated that siRNA knockdown of MAVS in 293T cells reduced the levels of apoptosis induced by reovirus infection. This finding was strengthened by work in $MAVS^{-/-}$ mice treated with the synthetic dsRNA Poly(I:C) following exposure to cigarette smoke. Researchers found that the combination of viral insult and exposure to tobacco smoke led to an increase in caspase-3 activation and apoptosis, and that this outcome was enhanced by the presence of MAVS [32]. From these results it would appear that MAVS may be pro-apoptotic, though this may only be true under certain conditions, such as acute infection.

As noted above, several viruses target MAVS for cleavage during infection as a means of abrogating interferon signalling. Many viruses, such as cytomegalovirus and human immunodeficiency virus-1, also target the host cell's apoptotic machinery as a means of preventing an immune response, thereby aiding viral replication [33]. The RLH protein MDA-5 is cleaved during poliovirus infection, and also when cells are treated with the apoptosis-inducing chemical puromycin [34]. Interestingly, both conditions produce similar cleavage products and can be blocked by the caspase inhibitor zVAD-fmk, indicating that poliovirus infection uses endogenous host-cell caspases to degrade MDA-5 [34]. These data demonstrate that members of the RLH pathway are targeted by viruses through apoptosis, and two recent reports have linked MAVS to a similar process. Scott and Norris [35] investigated the behaviour of MAVS during the early stages of apoptosis, and found that it was cleaved in cells treated with staurosporine and actinomycin D. This cleavage, which could be blocked by overexpression of the anti-apoptotic protein Bcl-xL or incubation with caspase inhibitors, removed MAVS from the outer mitochondrial membrane, in a manner similar to cleavage by HAV and HCV [35]. In a contemporary study, Rebsamen et al [20] showed that the cleavage of MAVS was carried out by an unidentified caspase at Asp429, just one amino acid from the HAV cleavage site at Gln428. Importantly, the study also showed that this cleavage abrogated the signalling capacity of MAVS when the fragments were expressed exogenously [20]. However, while it is clear that MAVS and MDA-5 are targets of apoptotic cleavage, further work is required to show that this is of functional benefit to the invading virus. For example, while poliovirus infection triggered the cleavage of MDA-5, this did not lead to an increase in viral replication or decrease in interferon production [34]. Despite this, the link between MAVS and apoptosis looks to be an important area for further research.

6. Other mitochondrial proteins involved in the innate immune response

Following the identification of MAVS as a key component of the intracellular pathway of the innate immune system, three other mitochondrial proteins have been implicated in the antiviral response – either as regulators of MAVS function, or as independent stimulators of interferon production. NOD-like receptor family member X1 (NLRX1, also known as NOD9 and CLR11.3) is a nucleotide-binding domain (NBD) and leucine-rich repeat (LRR) domain-containing protein that is localized to the mitochondrial compartment through a putative N-terminal targeting sequence [36]. NLRX1 was shown to disrupt RLH pathway signalling to NK-κB and IFN-β promoters by interacting with MAVS. Domain-deletion and co-immunoprecipitation experiments showed that the N-terminal NBD of NLRX1 interacted with the CARD domain of MAVS, thereby limiting its ability to bind to RIG-I following infection [36]. Finally, siRNA reduction of NLRX1 levels increased RLH signalling, which led the authors to conclude that this protein was a negative regulator of MAVS function, possibly as a brake on deleterious and prolonged interferon production [36].

Published contemporaneously with this study, a second group reported an innate immune function of NLRX1, but with a notably different mode of action. Tattoli et al [37] again reported that NLRX1 localized to mitochondria, confirming that this is the first member of the NOD family to target this cellular compartment. As with Moore et al [36], the N-terminal portion of NLRX1 was required for mitochondrial localization and function, and prevented the direct activation of NF-κB-promoter reporter constructs [37]. However, in contrast with the first group, NLRX1 was able to mount a possible immune response by acting as a potentiator of mitochondrial ROS production, which is a known inducer of pro-inflammatory signalling. This was demonstrated by an ability to increase ROS and activate the pro-inflammatory kinases NK-κB and JNK within minutes of either *Shigella* infection or TNF-α treatment [37].

The function of NLRX1 (as both an inhibitor and stimulator of NK-κB) during the innate immune response, as reported by these two studies, may be hard to reconcile. While the idea of NLRX1 as a brake on prolonged expression of immune proteins [36] is valid, and indeed well-supported by their results, it runs counter to the NK-κB-stimulatory effect of this protein during *Shigella* infection [37]. As noted by Meylan and Tschopp [6], it may be that NLRX1 acts first as a suppressor of the immune response, before promoting it when conditions change. Further work on the kinetics of NLRX1 activation and signalling, along with its interaction with MAVS, should identify to what extent this NOD family member is a bifunctional regulator of the innate immune system.

The most recent report of a mitochondrial regulator of MAVS function is gC1qR (also know as p32 and C1QBP), a receptor for the globular head component factor C1q. Exogenous expression of gC1qR was shown to inhibit the RIG-I- and MDA-5-dependent activation of ISRE, NK-κB and IFN-β promoter constructs following sendai virus infection or poly(I:C) stimulation [38]. Conversely, siRNA knockdown of gC1qR increased ISRE and IFN-β levels in cells infected with sendai virus, or overexpressing RIG-I or MAVS. The authors show by co-immunoprecipitation and co-localization studies that this regulation is mediated through a MAVS-gC1qR binding interaction, which occurs on the outer mitochondrial membrane following viral infection [38].

While these data are encouraging, they are perhaps difficult to reconcile with previous reports on gC1qR, when viewed in terms of a putative MAVS interaction. Several studies have shown that mature gC1qR is located in the mitochondrial matrix, which it reaches following import from the cytosol and cleavage of an N-terminal mitochondrial localization sequence (which removes the first 73 amino acids from this 282 residue protein [39,40]). This localization would keep it well away from MAVS under normal physiological conditions. The authors speculate

that an unidentified subset of gC1qR is resident in the cytosol, and that this may translocate to the mitochondria under infection conditions; indeed, they show that a truncated, mature form of gC1qR (lacking the mitochondrial localization sequence; $gC1qR^{74-282}$) can translocate to the mitochondria following incubation with sendai virus when ectopically expressed [38]. However, for the truncated form to exist, the full length protein ($gC1qR^{1-282}$) would presumably have to be imported into the mitochondria, cleaved of its mitochondrial localization sequence, and then exported back to the cytosol. Here it would remain until infection, at which point it would translocate back to the outer mitochondrial membrane to regulate MAVS signalling. While mitochondrial proteins are released under stressful conditions (for example, cytochrome *c* and Smac/DIABLO during apoptosis), this would be appear to be a unique, and particularly complex, pathway. In a similar manner to NLRX1, more work is required to establish the exact function of this protein as a negative regulator of MAVS in the fine-tuning of the innate immune response.

The final mitochondrial-associated regulator of the innate immune system is Mediator of IRF3 Activation or MITA (also known as MPYS and STING, due to simultaneous identification by three different groups). First reported as a mitochondrial- and plasma membrane-localized protein associated with the type II major histocompatibility complex and subsequent apoptosis (MPYS; [41]), MITA was identified by Zhong et al [42] during a screen of human spleen cDNAs capable of activating a reporter construct for ISG54. Subcellular fractionation and confocal analysis of overexpressed MITA showed that it was localized to the outer mitochondrial membrane, where it was postulated to connect MAVS to the downstream signalling kinases TBK-1 and IRF3 [42]. Knockdown of MITA lowered sendai virus-induced activation of IFN-β and NK-κB, which in control cells was initiated by TBK1-mediated phosphorylation of MITA [42]. At the same time, a third group reported that STING, a product of the same gene that encodes MPYS and MITA, was involved in the innate immune response [43]. While the reports on MITA and STING shared some key features (for example, (1) the ability to activate ISRE reporter constructs by overexpression; (2) that the protein was required for antiviral signalling, and its knockdown abrogated the innate immune response; (3) that it connected the RLH proteins to downstream kinases), they were perhaps more notable for their differences. Importantly, while MITA was localized to mitochondria, STING was shown (using similar methods) to be an endoplasmic reticulum protein [42,43]. Additionally, it was reported that while STING could activate NK-κB, ISRE and IFN-β reporter constructs, data presented in the MITA paper showed a positive activation of ISRE only, despite the other two reporters being tested [42,43]. While it is clear that the discovery of MITA/STING is important to our understanding of the regulation of innate immune signalling in general, and the RLH pathway specifically, much work is still required to untangle these contradictory results.

7. Conclusion

The identification of MAVS as a member of the RIG-I-like helicase pathway marked a new and exciting step in antiviral research, placing mitochondria at the forefront of the innate immune response. As the function of MAVS and its regulation continues to be elucidated, it is clear that we still have much to learn about this unique protein. One important question that still needs to be addressed is: “Why is MAVS localized to mitochondria”? In their initial paper Seth et al [9] demonstrated the requirement for MAVS to be attached to the mitochondrial outer membrane for full activity, even when it is directed there by the mitochondrial targeting sequence of other proteins. One answer may be that MAVS requires a membrane attachment, and that mitochondria provide a convenient scaffold upon which signalling proteins can be directed to maximize efficiency. However, it is clear that any intracellular membrane will not fit the purpose, as MAVS mislocalized to the ER or plasma membrane has greatly impaired function [9]. From this evidence, it would appear MAVS is located on the mitochondrial outer membrane for a particular reason, and this may be linked to the function of mitochondria

themselves. It is possible that MAVS, in addition to signalling in the interferon pathway, may communicate with mitochondria during infection so that they may change their energetic or biochemical state; changes which may aid the innate immune response in ways that we currently do not appreciate.

Another important area for future research is likely to be where MAVS sits in the evolution of the mammalian innate immune system. While mitochondria are common to plants, animals and yeast, their involvement in immunity has only been conclusively demonstrated so far in higher mammals. Indeed, MAVS shares no apparent sequence homology with any protein in *Arabidopsis thaliana*, *Saccharomyces cerevisiae* or *Drosophila melanogaster*, indicating that this system has developed later in the evolutionary spectrum. This is in contrast to other immune protein families, such as those containing leucine-rich repeat domains, which appear more ancient and are found in plants and animals [1]. Attempting to find out how, and at what point, mitochondria became involved in the mammalian antiviral response may provide us with a greater understanding of many aspects of immunity.

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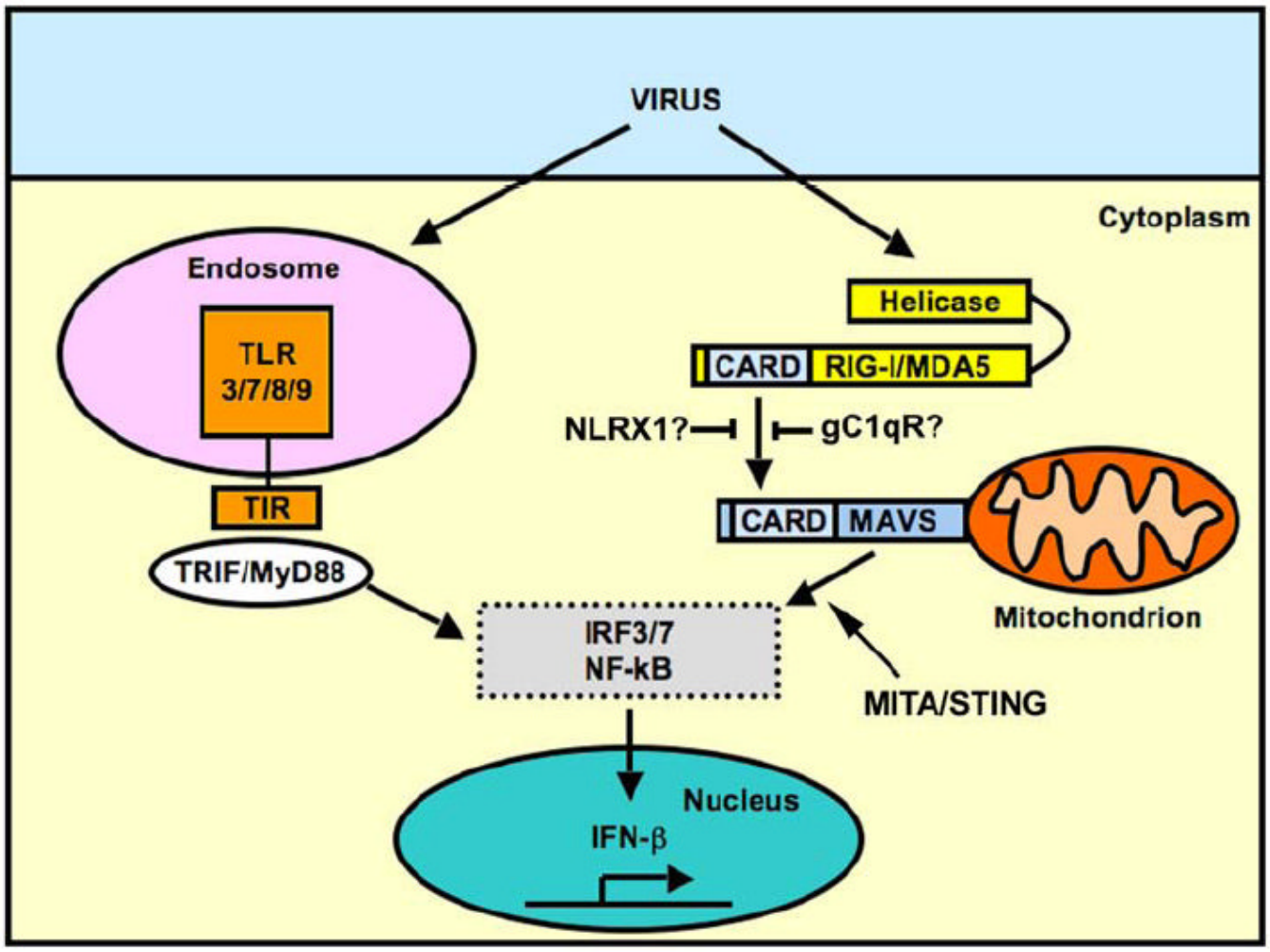

Figure 1. Overview of innate immune response signalling pathways

The response to extracellular pathogenic components are coordinated by pattern-recognition receptors (PRRs) such as Toll-like receptors (TLRs), represented here by the endosomal TLRs 3, 7, 8 and 9. Components are internalized from the cell surface in endosomes, and specific ligands (e.g. dsRNA, ssRNA, umethylated CpG DNA) are recognized by TLRs. The TLRs signal to cytoplasmic adapters such as TRIF and MyD88 through their TIR domain, which in turn control the activation of transcription factors (here represented by IRF3/7 and NF-kB) that lead to the production of type 1 interferons and pro-inflammitory cytokines. Intracellular pathogenic components, such as dsRNA and dsDNA, are recognized by the cytosolic PRRs, represented here by the DEAD-box RNA helicases RIG-I and MDA-5. Binding of viral nucleotides to these helicases induces a conformational change to expose the N-terminal caspase activation and recruitment (CARD) domain, which can then interact with the CARD domain on MAVS. This CARD-CARD interaction, which may be blocked by NLRX1 or gC1qR, induces a signalling cascade that activates pro-cytokine transcription factors and the production of type 1 interferon.

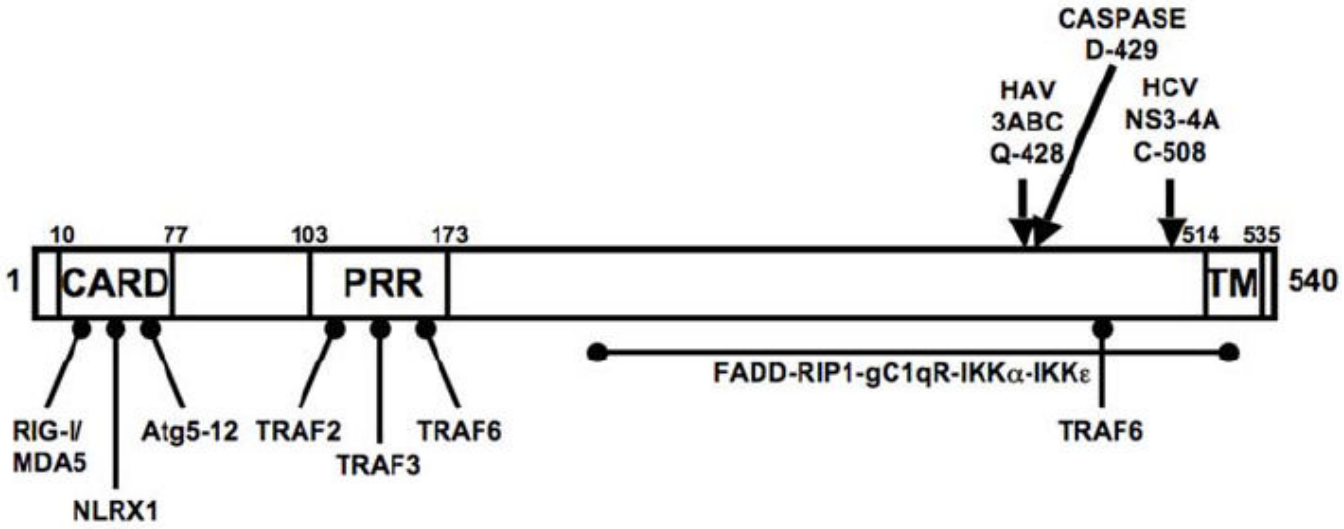

Figure 2. MAVS structure and interaction sites

The 540 amino acid MAVS protein consists of a N-terminal caspase activation and recruitment (CARD) domain, a medial proline-rich region (PRR) and a C-terminal transmembrane (TM) which anchors it to the outer mitochondrial membrane. Cleavage sites (top) and protein:protein interaction sites (bottom) are marked. Top - Hepatitis A (HAV) and C (HCV) viral proteases, along with an unidentified caspase, cleave MAVS from the outer mitochondrial membrane near the C-terminus. Bottom – Numerous MAVS-interacting proteins have been identified, including CARD proteins such as RIG-I and MDA-5, TNF-receptor associated factors (TRAFs) and NF-kB-inhibtor-related kinases (IKKs), Known interaction sites are marked with a circular arrow, whereas undefined sites are listed below the bar.