IRF-3 partners Bax in a viral-induced dance macabre

James E Vince and Jurg Tschopp*

Department of Biochemistry, University of Lausanne, Epalinges, Switzerland

*Correspondence to: jurg.tschopp@unil.ch

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In this issue of *The EMBO Journal*, Chattopadhyay *et al* (2010) describe a surprising new mechanism for how viral dsRNA detection by the RIG-I/MAVS signalling complex can initiate apoptosis. Independent of its transcriptional function, a pool of interferon regulatory factor (IRF)-3 activated downstream of MAVS can bind to and activate cytosolic Bax, resulting in Bax translocation to the mitochondria and initiation of the intrinsic apoptotic pathway.

The essential requirement of the mitochondria localized protein MAVS (also known as IPS-1, VISA or Cardif) to orchestrate the innate immune response following the detection of various cytoplasmic RNA molecules by the RNA helicases RIG-I or Mda5 is well established (Yoneyama and Fujita, 2009). MAVS-dependent signalling results in the activation of the transcription factors NF- κ B and IRFs, such as

IRF-3, which can act separately to induce inflammatory cytokines and chemokines or act together as part of an enhanceosome to induce type I interferons (IFNs).

More recently, several groups have suggested that MAVS can also function in apoptosis induction to limit viral replication and spread. Activation of RIG-I, and subsequent downstream MAVS signalling, can induce transcription of the pro-apoptotic BH3-only protein Noxa (and Puma) independent of p53, resulting in activation of the intrinsic apoptotic pathway, whereby Bax and Bak function to disrupt mitochondrial membrane integrity leading to cytochrome C release and formation of the caspase-9 apoptosome (Figure 1A) (Besch *et al*, 2009). Consistent with a role for IRF-3 in mediating Noxa transcription, activated IRF-3 binds to IRF-E elements within the Noxa promoter (Lallemant *et al*, 2007) and IRF-3 knockout or knockdown cells are refractory to

Figure 1 Models for MAVS-induced apoptosis. (A) IRF-3 transcription model. dsRNA transfection or that generated from replicating viruses such as VSV and SeV, binds RIG-I leading to MAVS activation on the mitochondrial outer membrane. TRAF3 recruits TBK1 and IKK- β resulting in IRF-3 phosphorylation and activation. Homodimers of IRF-3 (or IRF-3/IRF-7 heterodimers) induce transcription of the BH3-only proteins Noxa and Puma. These antagonize pro-survival Bcl-2 proteins such as Mcl-1 and Bcl-xL leading to Bax and Bak activation and loss of mitochondrial membrane integrity. Cytochrome C release causes apoptosome formation and cell death. (B) IRF-3 direct killing model. MAVS signalling results in IRF-3 activity as in (A), except instead of translocating to the nucleus, a portion of activated IRF-3 now binds cytosolic Bax through a BH3-like domain. IRF-3-induced Bax activity results in the efficient induction of apoptosis. (C) Anokis model. Cell detachment results in apoptosis induction in many different primary cell types. DAP3, a proposed proponent in anokis induction, binds to MAVS on cell detachment. MAVS recruits FADD and caspase-8 resulting in caspase-8 activation. Caspase-8 can activate downstream effector caspases (not shown), or cleave Bid to produce activated tBid. tBid antagonizes pro-survival Bcl-2 family members allowing Bax and Bak induced cell death to proceed.

apoptosis induction by RNA viruses such as those from the Reoviridae family, Vesicular stomatitis virus (VSV) and Sendai virus (SeV) (Holm *et al.*, 2007; Peters *et al.*, 2008). Conversely, expression of constitutively active IRF-3 (but not IRF-7) can induce cell death independent of type I IFN production (Heylbroeck *et al.*, 2000).

Consistent with these earlier observations, Chattopadhyay *et al.* (2010) report here that SeV and dsRNA transfection result in RIG-I and MAVS-dependent apoptosis and that this is severely compromised when IRF-3, or its upstream activators TRAF3 and TBK1, are depleted or knocked out. However, both TRAF2 and TRAF6 knockout cells are also resistant to dsRNA transfection-induced death, even though IRF-3-dependent gene induction appears normal in these cells. In addition, when IRF-3-deficient cells are reconstituted with a series of IRF-3 mutants lacking transcriptional activity, they still undergo caspase-9-dependent apoptosis in response to dsRNA transfection or SeV challenge. These data clearly suggest a distinct activation mechanism for IRF-3 that can induce cell death independent of its transcriptional function. In a series of elegant experiments, the authors show that a BH3-like domain of IRF-3 mediates binding to cytosolic Bax (but not Bcl-2, Bcl-xL or Bak) to induce Bax activation, mitochondrial translocation and oligomerization, culminating in cytochrome C release and apoptosome formation (Figure 1B).

It is unclear at this stage whether on binding, IRF-3 displaces a pro-survival Bcl-2 protein from Bax, to allow Bax activation, or whether IRF-3 directly activates Bax. The *in vitro* experiments performed by Chattopadhyay *et al.*, using purified proteins, imply that IRF-3 directly activates Bax in a similar vein to the direct Bax activation model that has been proposed for the BH3-only proteins Bim, Bid and Puma (Giam *et al.*, 2008). How TRAF2 and TRAF6 contribute to the IRF-3-dependent apoptotic response remains unresolved. However, their role in RIG-I-induced NF- κ B and/or MAPK activation, and their E3 ubiquitin ligase activity, leaves open the possibility for either indirect or direct functions in IRF-3-dependent killing.

The apparent dual role of IRF-3 induction of cell death occurring by transcriptional-dependent and -independent mechanisms is not unprecedented. Although p53 promotes transcription of pro-apoptotic Bcl-2 family members, such as

Puma, to initiate apoptosis, it has also been proposed that p53, like IRF-3, can directly bind to and activate Bax independent of its transcriptional functions (Chipuk *et al.*, 2004). It will be interesting to determine whether, like suggested for p53, the BH3-like domain of IRF-3 can also act to displace and activate traditional BH3-only family members that can be sequestered by their pro-survival Bcl-2 family counterparts.

Three scenarios have now been reported in which MAVS signalling may lead to a cell's demise (Figure 1). Although all three result in the activation of the intrinsic apoptotic pathway, the third scenario occurs independent of IRF-3 function and is induced by anoikis, the process whereby the detachment of adherent cells from the extracellular matrix induces apoptosis. It was recently reported that cell detachment results in death-associated protein-3 (DAP3) binding to MAVS and activation of the extrinsic cell death regulators, FADD and caspase-8 (Figure 1C), and correspondingly, MAVS knockout cells were resistant to anoikis (Li *et al.*, 2009). Caspase-8 can activate effector caspases directly, or can cleave Bid to induce Bax activation, explaining the ability of pro-survival Bcl-2 protein expression to block anoikis in some circumstances.

In summary, the data presented by Saurabh Chattopadhyay and colleagues in this issue of *The EMBO Journal* add to the growing evidence that MAVS signalling can combat virus infection by numerous operandi. Moreover, their results imply that Bax activators need not be limited to the classical set of BH3-only proteins, but may extend to proteins traditionally associated with a single function such as transcription. It will be important in future studies to address the importance of transcriptional-dependent IRF-3 killing, such as the requirement for Noxa induction, versus transcriptional-independent IRF-3 killing, *in vivo*.

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Conflict of interest

The authors declare that they have no conflict of interest.

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