Unexpected roles for DEAD-box protein 3 in viral RNA sensing pathways

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Abbreviations

DEAD: Asp-Glu-Ala-Asp. DDX3: DEAD/H Box 3. IKK: IκB kinase: IPS-1: IFN-β promoter stimulator-1. IRF3: IFN regulatory factor-3. MDA5: melanoma differentiation-associated gene 5. RIG-I: retinoic acid-inducible gene-I. RLR: RIG-I-like receptor. TBK1: TANK binding kinase 1.

Abstract

Detection of viral nucleic acid within infected cells is essential to an effective anti-viral response. The retinoic acid-inducible gene-I (RIG-I)-like receptors (RLR) form part of the virus detection repertoire and are critically important in sensing viral RNA in the cytoplasm. Efforts continue to define the signalling components downstream of RLR that are required to induce type I IFN (IFN-α and IFN-β) after viral infection. One surprising finding was that the DEAD (Asp-Glu-Ala-Asp) box helicase DDX3 (DEAD/H Box 3), known for some time to have a number of roles in cellular RNA regulation in the nucleus, has a role in the RLR cytoplasmic signalling pathway involved in IFN-β induction. In this issue of the *European Journal of Immunology*, an article reports an additional distinct positive role for DDX3 in the RLR RNA sensing pathway. This further emphasises the importance of DDX3 in anti-viral immunity, and is consistent with the idea that viruses target DDX3 for immune evasion.

The innate immune system relies on pattern recognition receptors (PRR) to recognise pathogen-associated molecular patterns (PAMP) and damage-associated molecular patterns (DAMP) [1]. For effective anti-viral immunity, is essential for host PRR to recognise and mount an appropriate response to non-self nucleic acid, such as that from viruses. One goal of PRR recognition of nucleic acid is the activation of the type I IFN system, through which an appropriate anti-viral immune response ensues [2].

The retinoic acid-inducible gene-I (RIG-I)-like receptors (RLR) RIG-I and melanoma differentiation-associated gene 5 (MDA5) serve as cytoplasmic PRR of viral RNA

[2]. Both RIG-I and MDA5 signal through a mitochondria-associated adaptor called IFN-β promoter stimulator-1 (IPS-1; also known as mitochondrial anti-viral signalling protein, Cardiff or virus-induced signalling adaptor). IPS-1 then signals the kinases TANK-binding kinase 1 (TBK1) and IκB kinase ε (IKKε) to phosphorylate IFN regulatory factor-3 (IRF3) and IRF7, resulting in the induction of type I IFN.

In this issue of the *European Journal of Immunology*, Oshiumi *et al*. [3] identify DDX3 as a component of the IPS-1 complex, and a positive regulator of IPS-1-mediated IFN-β induction. Like RIG-I and MDA5, DDX3 (also known as DBX) is a member of the DEAD-box family of RNA helicases. Members of this family contain an Asp-Glu-Ala-Asp (DEAD) motif, which is predictive of a role in RNA binding and RNA-dependent cellular processes [4]. DDX3 is ubiquitously expressed in a variety of cells, consists of 662 amino acids, and contains the central core helicase domain that typifies the DDX family [5]; however, the C- and N-terminal regions of DDX3 are distinct from other DDX proteins [6]. DDX3 appears to be involved in almost every step of RNA metabolism in the cell, and a role for DDX3 in cell cycle control and apoptosis has been proposed [4].

The findings in this issue that DDX3 has a positive role in IFN induction [3] are consistent with two previous reports. Schroder *et al.* [7] showed that the vaccinia virus protein K7 inhibited PRR-induced IFN-β promoter induction by binding to DDX3, which led to the discovery that DDX3 had a positive role in the RLR pathway. In Schroder *et al.*'s study [7], a virus-induced interaction between DDX3 and IKKε was observed, and further evidence indicated that DDX3 exerts its positive effect as part of the TBK1- and/or IKKε-containing complex(es) that activate IRF3 [7].

Interestingly, the positive role of DDX3 in IRF3 activation mapped to the N-terminus of DDX3, *i.e.* the same region of the protein targeted by K7 for IRF3 inhibition. Crystral structure analysis of K7 in complex with a peptide from the DDX3 N-terminus [8] has since confirmed this finding. Independently, Soulat *et al.* [9] also demonstrated a positive role for DDX3 in IFN-β promoter induction. Those authors showed synergistic activation of the IFN-β promoter by DDX3 and TBK1 and that DDX3 is a kinase substrate for TBK1. Furthermore, DDX3 was shown to be recruited to the IFN-β promoter [9]. Together, these findings placed DDX3 as a positive regulator of the RLR-induced IFN pathway at or below the level of the kinases TBK1/IKKε.

In contrast, the role for DDX3 in RLR signalling identified in this issue by Oshiumi *et al*. [3] seems somewhat distinct from these previous studies. Here, DDX3 is identified as an IPS-1-interacting protein via yeast-2-hybrid. The authors confirm DDX3 and IPS-1 interaction *via* overexpression studies in HEK293FT cells [3]. The results suggest this interaction to be constitutive [3], in contrast to the virus-dependent interaction between DDX3 and IKKε [7]. Furthermore, ectopically expressed DDX3 also interact with RIG-I and MDA5, and RIG-I- or MDA5-induced IFN-β promoter reporter gene activity was inhibited by DDX3 siRNA [3]. The authors [3] also demonstrated a synergistic activation of the IFN-β promoter by DDX3 and IPS-1. Importantly, this ability of DDX3 to activate the IFN-β promoter in the presence of IPS-1 was mapped to the C-terminal region of DDX3 (Figure 1). The authors [3] also demonstrate a direct anti-viral effect of DDX3 since upon its overexpression, viral replication was reduced in HeLa cells.

From these findings, the authors propose a model in which DDX3 serves as an initial sensor of RNA in unstimulated cells and that DDX3 intensifies signalling from IPS-1 when expression levels of RIG-I are low (Figure 1). The authors provide evidence for a role of DDX3 in direct RNA sensing by showing that DDX3 binds to polyI:C and viral RNA in solution [3]. While this data is not shown, there is however previous published data showing that DDX3 can bind various types of RNA [4]. Consistent with the idea that DDX3 is initially required to sense RNA before RIG-I is involved, the authors demonstration that upon polyI:C stimulation in HeLa cells, which express DDX3 constitutively, IFN-β mRNA induction reaches a maximum at 3 hours at which time RIG-I levels are still increasing. Also, in HEK293 cells infected with vesicular stomatitis virus, IFN-β mRNA was induced at 6 hours at which time no RIG-I message was detected.

The discovery that DDX3 can interact with IPS-I, MDA5 and RIG-I is an exciting finding in the field of RLR signalling. One could postulate that this finding is simply in keeping with the previously discovered role for DDX3 as an adaptor for the TBK1/IKKε kinases, since IPS-1 recruits these IKKs after virus stimulation, and this could explain the effect of DDX3 siRNA on IFN-β induction and the anti-viral activity of overexpressed DDX3. However the requirement for the DDX3 N-terminus for the TBK1 adaptor role, in contrast to the role for the DDX3 C-terminus for the IPS-1 interaction, suggests that these two functions for DDX3 in RLR signalling are distinct. Furthemore, the IPS-1 interaction is constitutive (at least for overexpressed proteins) [3] while the DDX3/IKKε interaction is virus-induced [7]. Thus, it now looks like DDX3 may have three distinct roles in enhancing RLR-induced IFN-β, namely as a component of an IPS-1 complex [3], as part of a TBK1/IKKε complex

[7], and via direct binding to the IFN-β promoter [9] (Figure 1). The exact mechanisms through which DDX3 acts at these different levels remain to be determined, but these novel roles of the terminal regions of DDX3 are in keeping with what has been discovered for other DDX family members in terms of signal-specific gene regulation [10]. One exciting possibility is that DDX3 is involved in recruiting TBK1 and IKKε to IPS-1. This would be consistent with DDX3 binding the IKKs and IPS-1 *via* two distinct motifs, but would not explain why the DDX3 C-terminal alone enhances IPS-1-induced IFN-β [3].

Whether or not DDX3 is actually an initial sensor of viral RNA during viral infection is a question that requires further investigation. Studies involving depletion of RLR by siRNA at early time points of IFN-β induction, or the use of cells from RLR-knockout mice would prove fruitful to definitively ascertain whether RIG-I has a role in early IFN-β responses to RNA, since in the experiments reported here [3], RIG-I may still be present upon initial viral sensing, but simply undetectable. Studies employing cells lacking RLR have already determined the essential role of MDA5 and RIG-I in sensing specific viruses, but it is still possible that the RLR are necessary but not sufficient for viral sensing. It would also be of interest to ascertain whether the helicase activity of DDX3 is required for the positive effect on IPS-1, since this was not required for the positive effect on TBK1 [7, 9], but might be expected to have a role in RNA sensing leading to activation of IPS-1. Since RIG-I activates signalling after RNA binding via a caspase recruitment domain (CARD)-CARD interaction with IPS-1, it would also be necessary to postulate how a non-CARD domain-containing protein such as DDX3 would stimulate IPS-1 signalling after RNA binding.

Overall the report from Oshimui *et al.* [3], together with previous studies [7, 9], clearly demonstrates a critical role for DDX3 in RLR signalling and the anti-viral response. Interestingly, four distinct viruses have been shown to target DDX3, both in terms of subversion of the function of DDX3 to facilitate viral replication, and as noted above for vaccinia virus, for evading detection by the immune system [4]. Thus there is now strong impetus to dissect the exact mechanisms through which DDX3 functions in the RLR viral sensing pathway; an endeavour that may generate therapeutically useful information.

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Conflict of Interest

The authors declare no financial or commercial conflict of interest.

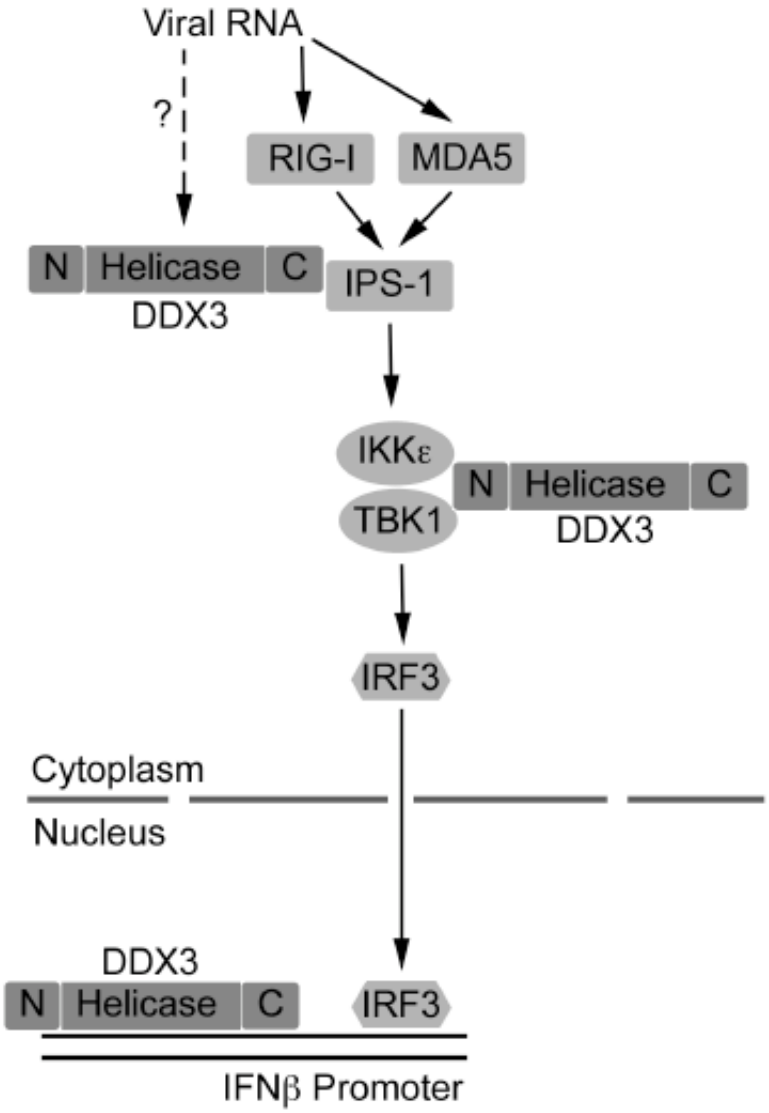

Figure 1

Multiple roles for DDX3 in viral sensing by RLR. Viral RNA binds to either RIG-I or MDA5 leading to recruitment of IPS-1 and downstream activation of the kinases TBK1 and IKKε, which are required for IRF3 phosphorylation and activation. IRF3 subsequently stimulates IFN-β induction. DDX3 has now been shown to have at least three functions in this pathway: enhancement of IPS-1 function via the C-terminus of DDX3 [3], as an adaptor for TBK1 and IKKε via the DDX3 N-terminus [7], and by directly binding to the IFN-β promoter [9]. Additionally, DDX3 is proposed to directly bind viral RNA during initial sensing of a virus by the RLR pathway.

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