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REVIEW

RNA helicase DDX3: at the crossroad of viral replication and antiviral immunity

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SUMMARY

Asp-Glu-Ala-Asp (DEAD)-box polypeptide 3, or DDX3, belongs to the DEAD-box family of ATP-dependent RNA helicases and is known to play different roles in RNA metabolism ranging from transcription to nuclear export, translation, and assembly of stress granules. In addition, there is growing evidence that DDX3 is a component of the innate immune response against viral infections. As such, DDX3 has been shown to play roles both upstream and downstream of I-kappa beta kinase ε (IKKε)/TANK-binding kinase 1, leading to IFN-β production. Interestingly, several RNA viruses, including human threats such as HIV-1 and hepatitis C virus, hijack DDX3 to accomplish various steps of their replication cycles. Thus, it seems that viruses have evolved to exploit DDX3's functions while threatening the innate immune response. Understanding this interesting dichotomy in DDX3 function will help us not only to improve our knowledge of virus–host interactions but also to develop novel antiviral drugs targeting the multifaceted roles of DDX3 in viral replication. Copyright © 2015 John Wiley & Sons, Ltd.

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INTRODUCTION

Asp-Glu-Ala-Asp (DEAD)-box polypeptide 3 (DDX3) belongs to a large family of ATP-dependent RNA helicases known as the DEAD-box family of proteins [1–3]. This family is characterized by the presence of a central catalytic core composed of 12 highly conserved motifs, including the eponymous DEAD motif II that provides the family's name [4,5]. DEAD-box proteins have been shown to be involved in all steps of RNA metabolism and thus are expected to serve pleiotropic functions within the cell [3].

DDX3 has two homologues, DDX3X and DDX3Y, located in chromosomes X and Y, respectively [6–8]. While DDX3Y is expressed in the male germline and is probably associated with male fertility [9], DDX3X (hence referred to as DDX3) is ubiquitously expressed in a wide range of tissues and is involved in key biological processes including cell cycle progression, innate immune response, apoptosis, and cancer but also the replication cycle of different viruses [1,8,10–12].

Most DDX3 functions are related to mRNA metabolism, mostly due to its ATPase and RNA helicase activities and its ability to interact with several proteins involved in this process [1,2] (Figure 1A). Interestingly, DDX3 is also an important component of stress granules (SG) [13,14], which are mRNA triage sites assembled during stress

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Abbreviations used

AIDS, acquired immunodeficiency syndrome; CARD, caspase activation and recruitment domains; CRM1, chromosome maintenance-1; DDX3, DEAD-box polypeptide; DEAD, Asp-Glu-Ala-Asp; DENV, dengue virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IFN, interferon; IKK, I-kappa beta kinase; IRF, IFN regulatory factor; JEV, Japanese encephalitis virus; LD, lipid droplets; LGP2, laboratory of genetics and physiology 2; MAVS, mitochondrial antiviral signaling; MDA5, melanoma differentiation-associated gene 5; MNV, murine norovirus; NV, norovirus; PABP, poly(A)-binding protein; PAMP, pathogen-associated molecular pattern; PKR, protein kinase R; PRR, pattern recognition receptor; RIG-I, retinoid acid-inducible gene-I; RLR, RIG-I-like receptor; RRE, rev responsive element; SG, stress granules; TBK, TANK-binding kinase; TLR, toll-like receptor; TRAF, TNF receptor-associated factors; VACV, vaccinia virus; UTR, untranslated region; WNV, West Nile virus.

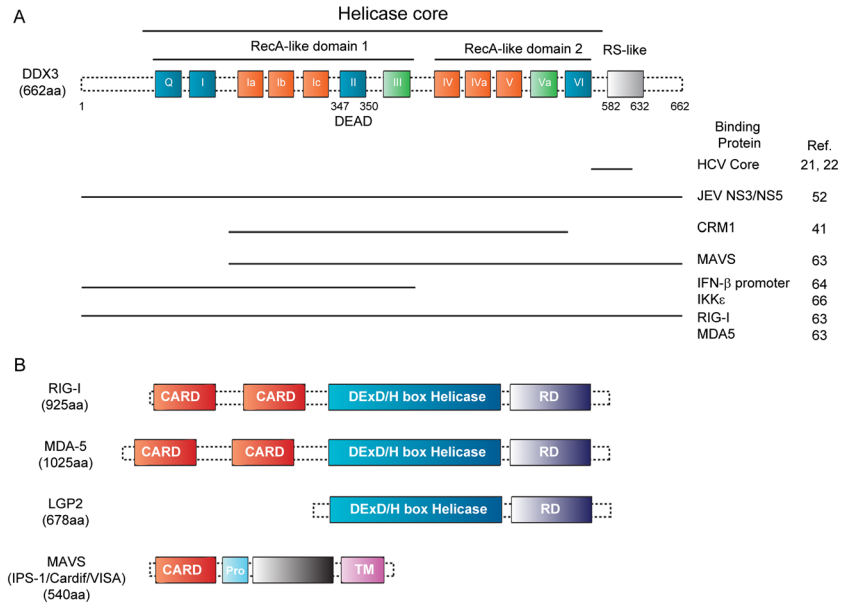

Figure 1. DDX3 and RLRs proteins in antiviral signaling. (A) Schematic representation of DEAD-box RNA helicase 3. The catalytic helicase core is composed of two RecA-like domains, domain 1 (encompassing helicase motifs Q, I, II, and III) and domain 2 (encompassing helicase motifs IV, V, and VI), both of which are essential to coordinate RNA binding and ATP hydrolysis activities. The C-terminal domain (CTD) contains an arginine-serine-rich (RS) domain. Motifs involved in ATP binding (blue), RNA binding (orange), and linking of ATP and RNA binding (green) are shown. The binding regions important for interaction with both viral and cellular proteins are shown. (B) Diagram illustrating key features of RIG-I, MDA5, and LGP2. The three RLRs are composed of a central DExD/H box helicase. A regulatory domain (RD) in the CTD binds viral RNA, activating the RIG-I ATPase by RNA-dependent dimerization. RIG-I and MDA5 contain tandem caspase activation and recruitment domain (CARD) regions at their N-terminal domain (NTD). A schematic representation of the MAVS/IPS-1/Cardif/VISA adaptor protein showing its CARD domain, the proline-rich region (Pro) and the C-terminal mitochondrial membrane region (TM) are presented. DDX3, DEAD-box polypeptide 3; RLRs, RIG-I-like receptors; DEAD, Asp-Glu-Ala-Asp; RIG-I, retinoid acid-inducible gene-I; MDA5, melanoma differentiation-associated gene 5; LGP2, laboratory of genetics and physiology 2; MAVS, mitochondrial antiviral signaling; VISA, virus-induced signaling adaptor; JEV, Japanese encephalitis virus

conditions and associated with innate immune responses against viruses [15,16]. Given the multifaceted functions of DDX3 in mRNA metabolism, it is not surprising that several RNA viruses exploit either the driving force of DDX3-mediated ATP hydrolysis or its ability to assemble large ribonucleoprotein complexes to accomplish different steps of the replication cycle [1,17]. Interestingly, growing evidence also supports DDX3 functions related to innate immune response against viral infection [12].

Improving our understanding of the roles of DDX3, both as a host factor for viral replication and as a component of the antiviral immune response, will contribute to the design of novel antiviral drugs aimed at fighting emerging and/or re-emerging viral diseases, such as HIV and HCV infections, which represent important threats to human health.

DDX3 as a common host factor required for RNA virus replication

DDX3 has been described as a host factor involved in the replication of several viruses with an important impact on human health (Table 1). In the following section, we describe the state of the art on this virus-DDX3 interaction.

Hepatitis C virus. HCV represents a significant human health burden, with more than 170 million people chronically infected worldwide [18]. Chronic HCV infection leads to steatosis, liver cirrhosis, and ultimately hepatocellular carcinoma [18]. HCV belongs to the *Hepacivirus* genus of the *Flaviviridae* family and possesses a positive single-stranded RNA molecule as a genome [19]. Early work using a yeast two-hybrid screening identified DDX3 as an interacting partner of the HCV core protein

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Table 1. DDX3 promotes RNA virus replication through multiple mechanisms

Virus	DDX3 function	Viral component	Cellular compartment
HCV	Late steps of viral replication, innate immunity evasion	Core, NSP5, NSP3, viral RNA	SGs and LDs
HIV-1	Nuclear export and translation of unspliced mRNA	Rev, viral RNA	Nuclear pore complex and pre-translation initiation granules
WNV	Viral replication, unknown mechanism	NS3	Cytoplasmic viral replication compartments
JEV	Viral replication, probably translation	NS3, NS5, Viral RNA	Cytoplasmic viral replication compartments
MNV	Viral RNA and protein synthesis	Viral RNA	Cytoplasmic viral replication compartments

DDX3, DEAD-box polypeptide; SG, stress granules; LDs, lipid droplets; WNV, West Nile virus; JEV, Japanese encephalitis virus; MNV, murine norovirus.

[20–22]. This interaction occurred between the N-terminal domain of core and the C-terminal arginine-serine-rich-like domain of DDX3 [21,22] (Figure 1A). Moreover, it was shown that expression of the HCV core protein in cells induced a strong redistribution of cytoplasmic DDX3 towards viral replication sites located around lipid droplets (LDs) [23–25]. Consistent with a functional interaction between DDX3 and core, a genome-wide screening identified DDX3 as a host factor involved in the late steps of the replication cycle [26]. Indeed, DDX3 was shown to be required for HCV RNA accumulation during viral replication [24,25]. However, while one study suggested that the DDX3 function was unrelated to its ability to interact with the core protein [23], other study showed that DDX3-binding peptides, derived from the HCV core N-terminal domain, were inhibitory for RNA replication of genotype 1b but not genotype 2a [27]. Thus, the relevance of core-DDX3 interaction during HCV replication still remains elusive, and more studies are needed. Although it remains unclear whether DDX3-related ATPase or RNA helicase activities are required for HCV replication, it has been proposed that DDX3 sequestration to HCV replication compartments around LDs contributes to evasion of innate immune responses [28] (see succeeding texts). Interestingly, DDX3 sequestration to LDs occurred together with the recruitment of several proteins including DDX6, Lsm1, Xrn1, PatL1, and Ago2, which usually localize in cytoplasmic granules including processing bodies (p-bodies) and SG [29]. Therefore, disruption of host cytoplasmic granules is an important step in ensuring viral replication (see succeeding texts).

Human immunodeficiency virus. HIV is the etiological agent of AIDS [30,31]. Since the beginning of AIDS epidemic, the virus has infected nearly 80 million people, with 40 million deaths. Despite the availability of antiretroviral treatment, it is not possible to eradicate the virus from the body, and thus, AIDS is now considered a non-curable chronic disease that requires life-long treatment [32]. HIV belongs to the *Lentivirus* genus of the *Retroviridae* family and possesses a positive single-stranded RNA genome that is retrotranscribed to a double-stranded DNA and integrated into the host cell genome [33]. Once integrated, the host RNA polymerase (Pol) II transcribes a 9-kb full-length mRNA molecule that undergoes full and partial alternative splicing to generate more than 40 viral transcripts [34]. In addition to these spliced transcript variants, the full-length unspliced transcript is also used as a mRNA [35,36]. While nuclear export of fully spliced transcripts relies on the classical cellular mRNA export machinery mediated by Tap/NXF1 [37], nuclear export of partially spliced and unspliced transcripts requires the "regulator of expression of virion proteins" known as the Rev protein [38,39]. Rev binds to a specific RNA

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structure present in viral mRNAs (Rev responsive element, RRE) and interacts with the host karyopherin (chromosome maintenance-1) CRM1, which finally mediates the nuclear export of the Rev/RRE complex [40]. Initially, DDX3 overexpression was shown to stimulate gene expression from an HIV-1 RRE-containing vector in the presence but not in the absence of Rev [41]. The authors observed that stimulation of gene expression from the RRE-containing mRNA was dependent on the catalytic activity of the RNA helicase and involved an increase in the cytoplasmic levels of the corresponding transcript; DDX3 was proposed to be a Rev co-factor during nuclear export of RRE-containing mRNAs [41]. Afterwards, DDX3 was also shown to directly interact with CRM1 at the cytoplasmic side of the nuclear envelope suggesting that the function of DDX3 was related to the late events of viral mRNA nuclear export [41]. DDX3 has also been shown to promote HIV-1 translation in an ATP-dependent manner [42–45]. While we and others identified the *trans*-activation response (TAR) RNA motif, present at the 5′-untranslated region (5′-UTR), as the molecular target of DDX3 during cap-dependent translation [42,45], it has also been proposed that DDX3 promotes cap-independent, internal ribosome entry site-driven, translation initiation [44]. During HIV-1 cap-dependent translation, we observed that DDX3 was necessary to (i) unwind the TAR RNA motif and (ii) drive the assembly of a specific ribonucleoprotein complex composed of at least the unspliced mRNA, DDX3, and translation initiation factors eIF4G and poly(A)-binding protein (PABP) [42,43]. Interestingly, such a pre-translation initiation complex was observed in the form of specific large cytoplasmic granules distinct from SG and p-bodies indicating that the relocalization of DDX3 to cytoplasmic viral aggregates might be a common strategy employed by RNA viruses [43] (see succeeding texts).

West Nile virus. West Nile virus (WNV) is a mosquito-borne virus infecting the central nervous system. Although most infected persons remain asymptomatic, symptomatic infection can lead to West Nile neuroinvasive disease manifested as encephalitis, meningitis, or acute anterior poliomyelitis, sometimes resulting in death [46]. WNV belongs to the *Flavivirus* genus of the *Flaviviridae* family and possesses a positive single-stranded RNA genome [47]. Interestingly, WNV disrupts the assembly of SG and p-bodies during infection [48]. As discussed previously for HCV and HIV-1, WNV sequesters DDX3 and other components of stress granules and p-bodies towards viral replication sites [49], further suggesting a common mechanism. Although it has been shown that DDX3 is required for viral replication [49], the underlying molecular mechanism remains unclear.

Japanese encephalitis virus. Japanese encephalitis virus (JEV) is also a mosquito-borne virus and the most common cause of encephalitis in humans, mainly affecting children [50]. Although most patients become immune following childhood infection, JEV causes nearly 70 000 infections annually [50]. Typically, 20–30% of infected patients die, and 30–50% of survivors suffer neuropsychiatric sequelae [50]. JEV belongs to the *Flavivirus* genus of the *Flaviviridae* family and possesses a positive single-stranded RNA genome [51]. DDX3 has been shown to be necessary for viral RNA translation and replication in an ATP-dependent manner during the JEV replication cycle [52]. Interestingly, DDX3 was able to interact with the viral proteins NS3 and NS5, components of the viral replication complex, as well as the 5′-UTR and 3′-UTR of the JEV RNA [52]. Indeed, DDX3 localized together with NS3 and NS5 and JEV RNA in cytoplasmic aggregates where viral replication takes place [52]. However, the molecular mechanism by which DDX3 promotes these interactions remains undetermined.

Norovirus. Norovirus (NV) is a human enteric pathogen and the first viral agent known to cause gastroenteritis. NV likely causes more than 200 000 deaths of children under 5 years old in the developing world annually [53]. Moreover, long-term chronic NV infection is now recognized as an important cause of morbidity and mortality in immune-compromised patients [54]. The virus belongs to the *Norovirus* genus of the *Caliciviridae* family and possesses a positive single-stranded RNA genome [53]. While there is no cell culture system for human NV, the use of murine norovirus (MNV) has provided a suitable model for studying NV replication [54]. Using RNA affinity chromatography followed by a mass spectrometry strategy aimed at characterizing the MNV

RNA-protein network, DDX3 was identified as a viral RNA-binding protein [55]. In addition, DDX3 knockdown resulted in a strong (nearly 90%) inhibition of viral RNA and protein synthesis as well as a reduction in viral titers, arguing for an essential role of DDX3 during viral replication. Similar to other RNA viruses described previously, DDX3 was observed forming cytoplasmic aggregates together with markers of the viral replication complex [55].

The type-I IFN-mediated antiviral innate immune response

More than 50 years ago, interferons (IFNs) were shown to inhibit viral replication [56]. Today, viruses and other pathogens such as bacteria and fungus are well known to contain molecular features or "patterns" absent in eukaryotic cells responsible for triggering IFN production [57]. These specific features, referred to as pathogen-associated molecular patterns (PAMPs), are recognized by pattern recognition receptors (PRR) present in host cells and allow for discrimination between self and non-self [57]. During viral infections, cell surface, endosomic, or cytoplasmic PRRs recognize viral PAMPs (cytoplasmic DNA, uncapped mRNA, and double-stranded RNA) and induce type-I IFNs (IFN-α and INF-β) [58]. Cytoplasmic DNA is mainly sensed by toll-like receptor 9 or by cyclic GMP-AMP synthase, which act through MyD88 and stimulator of interferon genes adaptor molecules, respectively [12,58]. As mentioned previously, viral RNA (either as an incoming genome or produced during viral replication) is the main RNA virus PAMP [58]. RNA helicases retinoid acid-inducible gene-I (RIG-I), melanoma differentiation-associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2) make up the RIG-I-like receptor (RLR) family (Figure 1B) and are the main cytosolic sensors of viral RNA [59–61]. RLRs have a similar structure composed of a central DExD/H-box helicase core domain and a C-terminal domain (CTD) that contributes to ligand specificity. In addition, RIG-I and MDA5, but not LGP2, contain two caspase activation and recruitment domains (CARDs) at the N-terminus, required for host cell signaling [60]. While RIG-I has been shown to be involved in sensing the *Paramyxoviridae* (Sendai virus and Newcastle disease virus), *Flaviviridae* (JEV and HCV), and *Rhabdoviridae* families (vesicular stomatitis virus), MDA5 detects members of the *Picornaviridae* family (poliovirus and encephalomyocarditis virus). Moreover, reovirus, dengue virus (DENV), and WNV are recognized by both RIG-I and MDA5 [58]. LGP2 has not been associated with any particular virus but rather acts as a positive regulator of the MDA5 signaling pathway [58].

The mitochondrial antiviral-signaling adaptor protein (MAVS, also known as IPS-1, Cardif, or virus-induced signaling adaptor) possesses an N-terminal CARD domain that interacts with the respective CARD present in RIG-I and MDA5 [58] (Figure 1B). MAVS also contains a proline-rich domain and a C-terminal transmembrane domain allowing for localization at the outer mitochondrial membrane, critical to achieving its functional properties [58] (Figure 1B). The RLR–MAVS interaction induces the recruitment of TNF receptor-associated factors (TRAFs) (Figure 2). TRAFs trigger the activation of cytoplasmic kinases, such as I-kappa beta kinase ε (IKKε)/TANK-binding kinase-1 (TBK-1) and IKKα/β, which phosphorylates and activates IFN regulatory factor 3 (IRF3)/7 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) transcription factors, respectively [12,57,62], leading to IFN-β production (Figure 2).

DDX3 is involved in type-I IFN-mediated antiviral innate immune response

In addition to RIG-I and MDA5, other RNA helicases, including DDX3, DDX60, DDX41, DDX1, and DHX9, have been recently linked to viral nucleic acid sensing and IFN-β production [12]. Interestingly, DDX3 has been shown to act both upstream and downstream IKKε/TBK-1 through the association with MAVS and IKKε/TBK-1 (Figure 2) [63–65]. DDX3 directly recognizes viral RNA and sensitizes the RLR pathway, probably when IFN-inducible RIG-I levels are below the threshold required to trigger downstream signaling (Figure 2) [63]. Alternatively, DDX3 also acts as a binding partner and a phosphorylation target of IKKε and TBK-1 (Figure 2) [64–66]. DDX3 binding to IKKε triggers the autophosphorylation and activation of the kinase. Then, activated IKKε phosphorylates several serine residues present in the DDX3 N-terminal domain, and the phosphorylated

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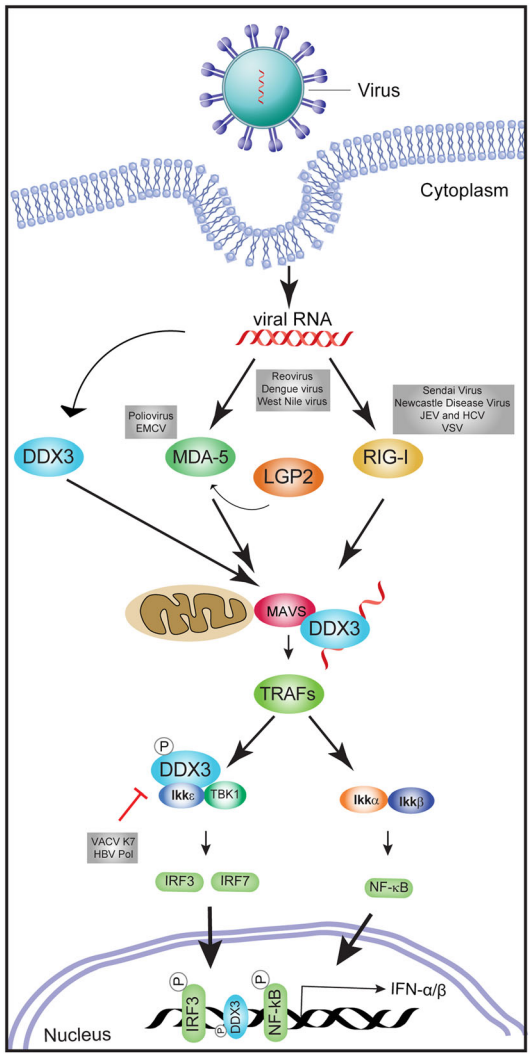

Figure 2. DDX3 is a member of the innate immune-response signaling pathway against viruses. Once the virus enters the cell, viral RNA is sensed by one of the RLR family members, RIG-I, or MDA5, supported by LGP2. RNA binding triggers RLR activation and the concomitant association with MAVS at the outer mitochondrial membrane. Then, MAVS activates the cytoplasmic kinases IKKε/TBK1 or IKKα/IKKβ leading to phosphorylation of IRF3/7 or NF-κB, respectively, and IFN production. DDX3 has been shown to (i) sense directly viral RNA and associate with MAVS; (ii) bind to IKKε to facilitate IRF3 phosphorylation, and (iii) being phosphorylated by TBK1 and bind the IFN-β promoter. All cases trigger IFN-β production and the innate antiviral response. Viral proteins such as VACV K7 and HBV Pol bind to DDX3 avoiding its interaction with IKKε/TBK1 and, as a consequence, IFN-β production. DDX3, DEAD-box polypeptide; RLR, RIG-I-like receptor; RIG-I, retinoid acid-inducible gene-I; MDA5, melanoma differentiation-associated gene 5; LGP2, laboratory of genetics and physiology 2; MAVS, mitochondrial antiviral signaling; IKK, I-kappa beta kinase; TBK, TANK-binding kinase; IRF, IFN regulatory factor; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; VACV, vaccinia virus; VSV, vesicular stomatitis virus; TRAF, TNF receptor-associated factors

DDX3 variant recruited IRF3 for its further phosphorylation by IKKε strengthening the signaling pathway [66]. On the other hand, phosphorylation of DDX3 by TBK1 has been involved in the direct recruitment of the helicase to the *ifnb* promoter leading to transcription activation [65]. In agreement with this, DDX3 has also been involved in the sensing pathway of viral dsDNA through the DNA-dependent activator of IRF protein (DAI) [67]. In this model, DDX3 also played a role downstream TBK1 through direct activation of the *ifnb* promoter [67]. An interesting feature of these different IFN-β-stimulating activities is the lack of ATPase and helicase activity requirement [65,67].

In contrast with its role as a host factor for viral replication, the ability of DDX3 to induce IFN-β has also been shown to interfere with replication of the vaccinia virus (VACV), hepatitis B virus (HBV), vesicular stomatitis virus (VSV), and dengue virus [63,64,68,69]. However, some of these viruses have evolved sophisticated mechanisms to target DDX3 and evade the induced innate immune response (Figure 2) [11].

Vaccinia virus, the live vaccine used for immunization against smallpox, belongs to the *Poxviridae* family. In sharp contrast with nuclear replicating DNA viruses, poxvirus replication cycle takes place in the cytoplasm of infected cells [70,71]. As such, VACV nucleic acids induce a strong innate immune response that is counteracted by viral proteins such as K7 that specifically inhibits IRF3/7 activation and IFN-β production through toll-like receptor-dependent and RLR-dependent pathways (Figure 2) [10,64,72]. Indeed, K7-deficient VACV has been shown to be less virulent and rapidly cleared compared with wild type virus, suggesting that K7 is a virulence factor regulating the innate immune response [73]. VACV K7 has the ability to interact with the DDX3 N-terminal domain [64,74,75]. Moreover, both IKKε and IRF3 bind to sites that overlap with the K7-binding site in the DDX3 N-terminal domain, and, thus, K7 interaction may interfere with DDX3-mediated IFN-β production [66]. Nevertheless, no direct evidence is available to show that DDX3 interferes with VACV replication, for example, in the context of a K7 mutant virus.

HBV is an important human threat and major cause of liver disease, with more than 400 million people persistently infected [76]. HBV belongs to the *Hepadnaviridae* family, with a dsDNA genome

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that undergoes reverse transcription during the replication cycle [77]. Specifically for HBV, DDX3 has been shown to bind to the viral polymerase (Pol), incorporates into virions, and interferes with reverse transcription and transcription [78,79]. Similar to what has been observed with VACV K7, HBV Pol interferes with both toll-like receptor-dependent and RLR-dependent pathways at the level of IKKε/TBK1-signaling molecules [68,80]. Moreover, the HBV Pol-DDX3 interaction interferes with the contact between the RNA helicase and IKKε leading to a reduced activation of IRF3 and further IFN-β induction (Figure 2) [68,80]. Of note, HBV Pol has been also shown to interfere with IFN-β production through NF-κB blockade [81]. The consequences of the HBV Pol-DDX3 interaction deserves further exploration.

As stated previously, DDX3 is required for HCV RNA replication probably throughout the interaction with the HCV core protein. Nevertheless, the mechanisms by which the HCV core protein modulates DDX3 actions on IFN-β production are still controversial. While binding of HCV core to DDX3 revealed a disrupted association between the RNA helicase and MAVS to block the DDX3-mediated induction of IFN-β production [28], another study indicates that HCV core-DDX3 interaction was required for IFN-β production [82]. Although a contact of core with DDX3 seems to be important for HCV RNA replication, it is still unclear whether the modulation of IFN-β production (positive or negative) mediated by protein–protein interaction has a real impact in viral replication.

DDX3 and cytoplasmic granules as components of antiviral immunity and viral replication

In addition to RLRs, viral RNA (such as dsRNA from replication intermediates) activates other antiviral molecules including protein kinase R (eIF2aK2 or PKR), 2′-5′-oligoadenylate synthetase (OAS), and RNAse L [83–85]. Of particular interest is PKR, which is activated upon RNA binding, promoting phosphorylation of the alpha subunit of the translation initiation factor eIF2 (eIF2α) [86]. This post-translational modification hampers guanosine diphosphate (GDP) to guanosine triphosphate (GTP) exchange in eIF2, leading to decreased ternary complex activity (eIF2/GTP/Met-tRNAi) and the concomitant suppression of translation initiation and SG assembly (Figure 3) [87]. Moreover, increasing evidence suggests that cytoplasmic granules, such as SG and p-bodies, are important players in the cellular response against viral infections [88,89]. SG and p-bodies are dynamic cellular microcompartments that contain different mRNA species and RNA-binding proteins [89–91]. While SG are translationally silent ribonucleoproteins assembled during stress conditions serving as storage sites for mRNAs and proteins, p-bodies are constitutively present within the cell and contain translational repressors, mostly components of the mRNA decay machinery [89–91]. Thus, inhibition of translation initiation through PKR-dependent eIF2α phosphorylation and SG assembly prevents viral replication by impeding viral protein synthesis [92,93]. However, viruses have evolved different mechanisms to modulate SG assembly to different levels in order to circumvent this antiviral system [92,93]. For instance, PKR, one of the most studied components of the IFN-induced antiviral pathway, localizes to SG during measles virus infection [94]. Relocalization of PKR to SG delays its activation and, as a consequence, its ability to induce the stress response [94]. Furthermore, master sensors of incoming viral RNA, RIG-I, MDA5, and LGP2 are also recruited to specific cytoplasmic granules, the antiviral stress granules (avSG), upon infection with an NS1-mutant influenza A virus [95]. The two other components of the antiviral system induced by IFN, OAS and RNAse L, have been also described in avSG [95] (Figure 3). Finally, the RIG-I activators, Riplet, TRIM25, and MEX3C ubiquitin ligases can also be recruited to SG [96,97]. Together, these data suggest that assembly of SG and/or avSG as a response to viral infection may be important in modulating the signaling pathway for IFN production.

As mentioned previously, DDX3 has been implicated in the early phases of viral RNA sensing, probably intervening with MAVS before activation of the RLR-dependent signaling pathway (Figure 2) [63]. In addition, DDX3 interacts with RIG-I, MDA5, TBK1, and IKKε and binds to the IFN-β promoter [28,63–66], demonstrating the multiple mechanisms by which the RNA helicase could modulate IFN production (see previous texts). Interestingly, DDX3 is a component of cellular cytoplasmic granules, including SG and p-bodies [1,13,14,42]. Of note, DDX3 overexpression triggers

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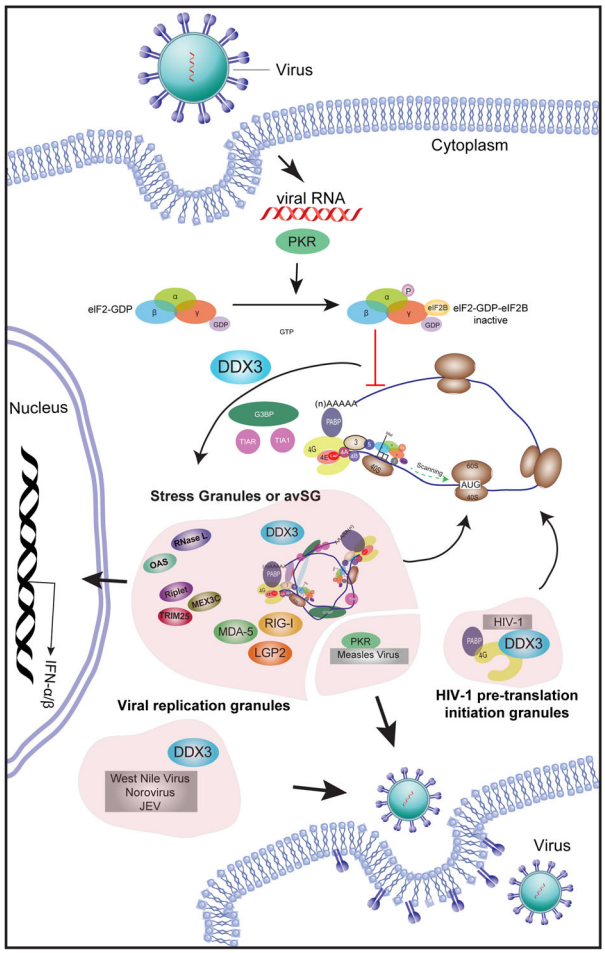

Figure 3. DDX3, cytoplasmic granules, and the viral replication cycle. One of the interferon-stimulated genes (ISG) involved in the antiviral response codes for the protein kinase PKR. In the cytoplasm, PKR binds to viral RNA (such as dsRNA from viral replication intermediates) and became activated triggering the phosphorylation of the alpha subunit of the translation initiation factor eIF2. Such a phosphorylation impedes the guanosine diphosphate (GDP)-guanosine triphosphate (GTP) exchange mediated by eIF2B leading to the formation of an inactive complex that blocks the initiation step of protein synthesis. Under this condition, mRNAs stalled in translation initiation associates with RNA-binding proteins such as TIA-1, TIAR, and G3BP1, which are nucleating factors that mediate the assembly of large cytoplasmic mRNPs known as stress granules (SG). Members of the RLR family and its activators (Riplet, TRIM25, and MEX3C), together with PKR and other ISG involved in the antiviral response (2′-5′-oligoadenylate synthetase (OAS) and RNase L), have been reported to localize in SG or antiviral SG (avSG) during viral infection indicating that assembly of SG participates in the cellular antiviral response. Similar to TIA-1, TIAR, and G3BP1, DDX3 was shown as a SG nucleating factor. RNA viruses including HIV-1, measles virus, WNV, JEV, and norovirus sequester DDX3 to specific viral replication compartments probably avoiding its ability to assemble in SG and induce INF-β production. DDX3, DEAD-box polypeptide; PKR, protein kinase R; RLR, RIG-I-like receptor; WNV, West Nile virus; JEV, Japanese encephalitis virus; PABP, poly (A)-binding protein

spontaneous SG assembly in the absence of any induced cellular stress, similar to what has been observed with the well-characterized SG-nucleating factors TIA-1, TIAR, and G3BP1 [14,98,99]. Although the molecular mechanism by which DDX3 induces SG formation remains unknown, the ability to interact with RNA and several translation initiation factors, such as PABPC1, eIF4G, eIF4E, and eIF4A, suggests that DDX3 may serve as a platform for the assembly of large macromolecular aggregates [13,14,42,43]. The ability of DDX3 to induce SG assembly has been shown to be independent of its ATPase and RNA helicase activities but dependent on an eIF4E-binding domain in the N-terminus, conferring DDX3 with self-aggregation properties [13]. On the other hand, DDX3 depletion results in nuclear accumulation of PABPC1, resembling the phenotype observed during rotavirus and herpesvirus infection in which PABPC1 relocalization prevents SG assembly to allow translation of viral mRNAs [100,101]. Thus, DDX3 seems to be an important component for regulating SG assembly. As mentioned previously, DDX3 has been described as a component of specific viral cytoplasmic granules containing the RNA of HCV [23,24,102], HIV-1 [43], WNV [49], JEV [52], and NV [55] (Figure 3 and Table 1). Recruitment of DDX3 to these structures suggests a potential shared strategy employed by RNA viruses to sequester the helicase, leading to its inability to assemble in SG and thus participate in the innate antiviral response. Consistent with this idea, viruses including HIV-1 [103], HCV [104], and WNV [48] interfere with SG assembly during their replication cycles [92,93]. Therefore, SG assembly, together with the recruitment of nucleic acid sensors, specialized in viral RNA sensing, to these structures may be critical to modulating IFN production and the innate immune response (Figure 3).

DDX3 connects cytoplasmic granules, viral replication, and the innate immune response

The dichotomic functions of cytoplasmic granules and its components, especially DDX3, in regulating the cellular response to viral infection while serving as a critical factor for viral replication started to be clarified during the last 2 years, at least, in the HCV model. As mentioned previously, DDX3 is redistributed to LDs upon HCV infection where the RNA helicase localizes together with the core

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protein [23,24,102]. Additionally, DDX3 has been shown to bind to the highly structured HCV 3 -UTR leading to IKKα activation [105]. Once activated, IKKα translocates into the nucleus inducing NF-κB-independent expression of lipogenesis-associated genes and LDs biogenesis, thus facilitating viral assembly [105] (Figure 4). Consistent is the requirement of IKKα for core protein association with LDs and thus, for the late steps of the viral replication cycle [105]. Moreover, DDX3 knockdown resulted in an inhibition of HCV 3 -UTR-induced LDs formation and core localization to these structures [105]. Furthermore, DDX3 has been demonstrated to drive the assembly of cytoplasmic aggregates, different to LDs, also accumulating IKKα and the HCV 3 -UTR [105] (Figure 4). Indeed, while DDX3 localized with the core protein around LDs, IKKα did not [105]. More recently, interactions between DDX3 and IKKα during HCV replication have been shown to localize in a dynamic way between two cytoplasmic compartments, SG and LDs [102]. *Per se*, upon HCV infection, DDX3 interacts with the HCV 3 -UTR and IKKα and drives the assembly of this complex in SGs [102] (Figure 4).

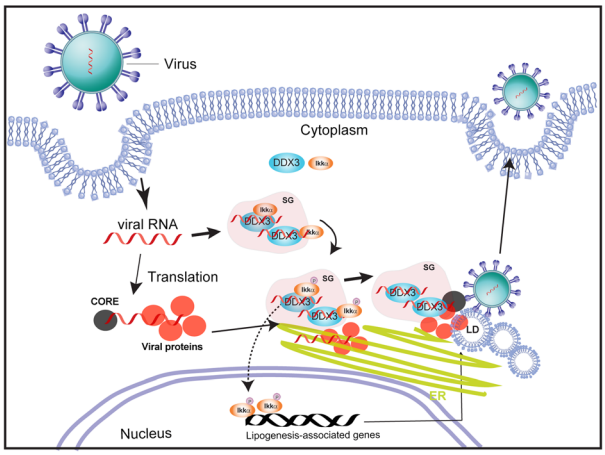

Figure 4. DDX3, cytoplasmic granules, and immune response during the HCV replication cycle. The HCV viral RNA is a major PAMP that triggers a strong immune response. However, DDX3 binds to the viral RNA and recruits IKKα forming a viral RNA/DDX3/IKKα complex that accumulates in SG, thus, avoiding its recognition by RLRs, PKR, or RNaseL. While IKKα is activated by phosphorylation and translocated to the nucleus to activate transcription of lipogenesis-related genes, the SG/DDX3/viral RNA complex (lacking IKKα) is redistributed to the surface of lipid droplets (LDs) where they join the HCV core protein to initiate the viral assembly. DDX3, DEAD-box polypeptide; PAMP, pathogen-associated molecular pattern; IKK, I-kappa beta kinase; RLRs, RIG-I-like receptors; PKR, protein kinase R; SG, stress granules

As infection proceeds and viral proteins accumulate, DDX3 and SG-associated proteins such as G3BP1 redistribute and localize together with the HCV core protein around LDs [102] (Figure 4). However, IKKα is not relocalized around LDs but rather translocates to the nucleus to stimulate LDs biogenesis [102,105] (Figure 4). These observations could help to explain the oscillation of SG assembly/disassembly detected in HCV-infected cells [104].

The complex program of interactions driven by DDX3 during HCV replication has several implications for our understanding in the interconnection between cytoplasmic granules assembly, viral replication, and innate immunity. Sequestering DDX3 and IKKα in SGs allows the potential inhibition of the innate antiviral response triggered by the HCV 3 -UTR, which was identified as a major HCV PAMP [106]. In parallel, SGs may also serve as sites of concentration of host proteins required for the late steps of viral replication. Consistent with this notion, SG assembly is necessary for HCV RNA replication, assembly, and egress [29,107,108].

CONCLUDING REMARKS

DDX3 is a multifaceted protein shown to participate in multiple physiological contexts most of them affecting human health. As different RNA viruses have evolved sophisticated strategies to sequester and exploit DDX3 during the viral replication cycle, pharmacological intervention involving DDX3 should be a potential target in developing antiviral drugs against human threats such as HIV and HCV. Small molecules targeting DDX3's catalytic activity have already been developed and tested in cell culture [109–111]. However, given the growing evidence for the catalytic-independent functions of DDX3 in important cellular processes such as innate immunity and cytoplasmic granule assembly, improving our understanding is critical to developing novel molecules that can interfere specifically in viral replication.

Although DDX3's involvement with the viral replication cycle was first reported at the end of the 90's, we are still far from understanding the molecular and cellular mechanisms at play. Recently described functions of DDX3 in innate immunity and cytoplasmic granule assembly invite us to continue our exploration of the mechanisms underlying the role of this fascinating DEAD-box protein.

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