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Commentary

Human DEAD-box protein 3 has multiple functions in gene regulation and cell cycle control and is a prime target for viral manipulation

Martina Schröder *

Host–Pathogen Interaction Laboratory, National University of Ireland Maynooth, Maynooth, Co.Kildare, Ireland

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ABSTRACT

The human DEAD-box RNA helicase DDX3 has been implicated to play a role in the whole repertoire of processes regulating gene expression, including transcription, splicing, mRNA export and translation. It has also been suggested to be involved in cell cycle control and the regulation of apoptosis. In addition, DDX3 was recently shown to be part of innate immune signalling pathways and to contribute to the induction of anti-viral mediators, such as type I interferon. Interestingly, DDX3 appears to be a prime target for viral manipulation: at least four different viruses, namely Hepatitis C virus (HCV), Hepatitis B virus (HBV), Human Immunodeficiency Virus (HIV) and poxviruses, encode proteins that interact with DDX3 and modulate its function. HIV and HCV seem to co-opt DDX3 and require it for their replication. It has therefore been suggested that DDX3 could be a novel target for the development of drugs against these two viruses, both of which still pose major global health threats. However, in the light of the apparent multifunctionality of DDX3 in the cell, drug development strategies targeting DDX3 will have to be carefully evaluated. This review summarises the available data on the cellular functions of DDX3 and discusses their manipulation by the different viruses known to target DDX3. Understanding the viral strategies for manipulating or co-opting DDX3 in functional and molecular detail can provide valuable insights for the development of strategies to therapeutically target DDX3.

1. Introduction

DDX3 (or DBX) is a member of the DEAD-box family of putative RNA helicases. It was first identified in 1997 as one of five X-chromosomal genes that have homologues in the non-recombining region of the Y-chromosome (DBY, DDX3Y) [1]. The DDX3 (or more correctly DDX3X) gene escapes X-inactivation [1] and is ubiquitously expressed in a wide range of tissues [2]. DEAD-box helicases are involved in a large variety of cellular processes involving RNA, such as splicing, mRNA export, transcriptional and translational regulation, RNA decay and ribosome biogenesis [3]. In recent years, DDX3 has received a lot of interest because of several studies showing its manipulation by viruses that pose major global health threats, such as Human Immunodeficiency Virus (HIV) [4], Hepatitis C virus (HCV) [5–8] and poxviruses [9]. Due to the finding that HIV and HCV seem to require DDX3 for their replication [4,8], the inhibition of DDX3 has been suggested as a novel therapeutic strategy for the development of drugs against these viruses [10]. However, it was recently demonstrated that DDX3 is also involved in the induction of anti-viral mediators [9,11] and it appears to function in protein translation, cell cycle control and apoptosis. Therefore, it seems crucial to understand how exactly DDX3 contributes to these processes in order to design informed strategies for its inhibition or manipulation. This review focuses in particular on the viral manipulation of DDX3 and discusses which cellular functions of DDX3 are targeted by the virus. In many cases, studying a viral protein and its host targets has provided novel insights about the function of the host target. For example, this was also the case with vaccinia virus protein (VACV) K7 which revealed a novel role for DDX3 in anti-viral immunity [9]. Therefore, it has been and will be a valuable approach to deduce information about DDX3 function from the way viruses are

Abbreviations: 4eBP, eIF4e-binding protein; aa, amino acid; Cdc, cell division cycle; CRM-1, chromosome maintenance region-1; DDX3, DEAD-box protein 3; eIF, eukaryotic initiation factor; EJC, Exon junction complex; GSK, glycogen synthase kinase; HBV, Hepatitis B virus; HCC, hepatocellular carcinoma; HCV, Hepatitis C virus; HIV, Human Immunodeficiency Virus; HLA, human leukocyte antigen; IAP, inhibitor of apoptosis; IFN, interferon; IkB, inhibitor of nuclear factor-kB; IKK, IB-kinase; IRES, internal ribosome entry site; IRF, interferon regulatory factorMAVS-mitochondrial antiviral signalling; mRNA, messenger RNA; NES, nuclear export signal; NPC, nuclear pore complex; NS3/4a, non-structural 3/4a; PABP, polyA-binding protein; PRR, pattern recognition receptor; RIG, retinoic-acid inducible gene; RLH, RIG-like helicase; RNP, ribonucleoprotein; rRNA, ribosomal RNA; siRNA, small interfering RNA; snRNA, small nuclear RNA; Sp1, specificity protein 1; TAP, tip-associated protein; TBK, TANK-binding kinase; TLR, Toll-like receptor; TRAIL-R, TNF-related apoptosis-inducing ligand-receptor; TRIF, TIR-domain containing adaptor inducing IFNβ; UTR, untranslated region; VACV, vaccinia virus.

* Correspondence address: Institute of Immunology, Biology Department, NUI Maynooth, Maynooth, Co.Kildare, Ireland. Tel.: +353 01 7086853; fax: +353 01 7086337.
E-mail address: martina.schroeder@nuim.ie.

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targeting it. This could lead to the development of therapeutic tools that mimic or prevent the viral strategy to manipulate DDX3 function (see Section 6).

2. Cellular localisation of DDX3

Several studies have described DDX3 as a protein that constantly shuttles between the cytoplasm and the nucleus, with its export from the nucleus being mediated by the export shuttle protein CRM1 [4,9,12]. CRM1 exports proteins containing a leucine-rich nuclear export signal (NES), and indeed DDX3 contains an NES within its N-terminal 22 amino acids (aa). However, the association between CRM1 and DDX3 does not seem to depend on this described NES [4] (discussed further in Section 3.3). More recently, it has been demonstrated that DDX3 can also be exported via the TAP-dependent export pathway, which normally mediates the nuclear export of mRNAs [13]. It remains to be demonstrated whether these two pathways are equally involved in nuclear export of DDX3, or whether one pathway is favoured over the other, possibly depending on the cell type or additional co-factors that associate with DDX3. Presumably due to the high rate of nuclear export, most studies investigating DDX3 localisation detected it mainly or exclusively in the cytoplasm [4,6,9,12–14]. DDX3 partially accumulated in the nucleus after inhibition of CRM1 [4,9,12,13] or TAP [13], suggesting that it can indeed be exported from the nucleus through both pathways.

In contrast to these results, two other studies found that endogenous DDX3 was mainly localised in the nuclei of untreated HeLa cells [5,7]. The reason for this discrepancy is unclear. However, Chao et al. also demonstrated that DDX3 is mainly nuclear in healthy primary epidermis cells, but largely cytoplasmic in skin tissue from cutaneous squamous cell carcinomas [15], suggesting a difference between transformed and non-transformed cells. It is conceivable that nuclear import and export of DDX3 are regulated; however the mechanisms for this have yet to be uncovered. For example, post-translational modifications could modulate either process, or DDX3 could be retained in the nucleus through the association with nuclear proteins. As the various described cellular functions of DDX3 include both cytoplasmic and nuclear processes (for example translational and transcriptional regulation, respectively), nuclear-cytoplasmic shuttling of DDX3 might be closely linked to its participation in these processes. In particular, DDX3 might be regulating or mediating nuclear export of mRNAs through its association with the nuclear export receptors (discussed in more detail in Section 3.3).

3. The role of DDX3 in RNA metabolism

DEAD-box helicases are involved in all aspects of RNA metabolism. Their role is thought to be the unwinding of RNA, i.e. the removal of secondary structure motifs, the unwinding of short RNA–RNA interactions and also the removal of RNA-bound proteins. All DEAD-box helicases contain nine conserved helicase motifs, including the eponymous Asp–Glu–Ala–Asp (D-E-A-D) motif, within a structurally conserved core element forming two recA-like domains. The conserved helicase motifs are involved in ATP binding, ATPase activity, RNA substrate binding and unwinding. However, the N- and C-termini of DEAD-box helicases are much more divergent and thought to confer functional specificity to individual DEAD-box helicases [3].

3.1. Unwinding of RNA

Ded1p, the yeast homologue of DDX3, appears to unwind RNA duplexes (and interestingly DNA–RNA duplexes) in a mode different from canonical translocating helicases [16]. Translocating helicases, e.g. DNA helicases, move along one strand of RNA or DNA directionally and in an energy-dependent manner. In the process, they displace complementary nucleic acid strands and/or interacting proteins.

However, Ded1p unwinds substrates without strict polarity. Based on this and other data, the authors proposed a mechanism by which the separation of the duplex is based on local destabilization of RNA helical regions, meaning that the helicase sitting on the duplex RNA 'switches' the two strands apart [16]. This would be a suitable mechanism for DEAD-box helicases, since they mainly appear to be involved in local structural changes of RNA and ribonucleoprotein (RNP) complexes, involving only a small number of base pairs [16,17]. It is possible that this mechanism distinguishes DEAD-box helicases from the related RNA helicases containing DExD- or DEAH-box motifs, some of which have been shown to be processive RNA helicases [17,18].

3.2. Splicing

Soon after its discovery the yeast Ded1p was linked to splicing of pre-mRNAs. However, it is still unclear whether Ded1p or mammalian DDX3 actually contribute to splicing [19]. It has been suggested that the C-terminus of DDX3 contains a region which resembles RS-domains found in splicing factors [5]. Ded1p and DDX3 appear to interact with the spliceosome and mRNPs [20,21] (Fig. 1A). However, DDX3 tightly associated only with spliced mRNAs in an Exon junction complex (EJC)-dependent manner [20]. This would suggest that DDX3 does not have an active role in splicing, but associates with RNPs after splicing, similar to proteins of the RNA export machinery.

3.3. Nuclear export of RNA

Several different nuclear transport receptors specifically mediate the export of proteins and the various classes of RNAs through the nuclear pore complex. As described in Section 2, DDX3 interacts with two of these export shuttle proteins: CRM1, the receptor that exports proteins containing a leucine-rich NES, and TAP, the main mRNA exporter (Fig. 1B). Ribosomal RNAs and small nuclear RNAs (snRNAs) are also exported in a CRM1-dependent manner [22].

The mRNA export shuttle protein TAP gets recruited to spliced mRNAs via the EJC (similar to what was shown for DDX3 [20]). The interaction between DDX3 and TAP involved the C-terminus of DDX3 (aa 536–661) [13]. The authors also demonstrated that DDX3 could be cross-linked to poly(A)-mRNAs in nuclear and cytoplasmic fractions. Binding of DDX3 to mRNAs was not disrupted by siRNA-mediated knock-down of TAP, nor did RNAse treatment disrupt the interaction between DDX3 and TAP [13]. Despite the finding that DDX3 binds to both, mRNAs and TAP, the authors were unable to show a contribution of DDX3 to polyA-mRNA export. It therefore seems unlikely that DDX3 is required for general mRNA export. However, it is still possible that DDX3 participates in the nuclear export of a specific subset of mRNAs via TAP.

The CRM1–DDX3 interaction is exploited by HIV which seems to 'tag' its incompletely spliced RNAs to the CRM1/DDX3 complex for export out of the nucleus. This is mediated via an interaction between the viral RNA-binding protein rev and DDX3 (Fig. 1B). Hence, DDX3 was shown to be required for the export of HIV RNAs from the nucleus [4] (further discussed in Section 5.5). However, the functional relevance of the CRM1–DDX3 interaction in uninfected cells remains unclear. Yedavalli et al. found that DDX3 was not required for CRM1-dependent export of proteins such as IkBα [4], therefore DDX3 does not seem to be a general cofactor of CRM1. Despite this, the authors postulated that DDX3 is