RNA Helicase A Regulates the Replication of RNA Viruses

Rui-Zhu Shi 1, Yuan-Qing Pan 1 and Li Xing 1,2,3,4,*

1 Institute of Biomedical Sciences, Shanxi University, 92 Wucheng Road, Taiyuan 030006, Shanxi province, China; 1549827168@qq.com (R.-Z.S.); pyqing0206@163.com (Y.-Q.P.)
2 Key Laboratory of Medical Molecular Cell Biology of Shanxi Province, Shanxi University, Taiyuan 030006, China
3 Shanxi Provincial Key Laboratory for Prevention and Treatment of Major Infectious Diseases, Shanxi University, Taiyuan 030006, China
4 * Correspondence: xingli107@gmail.com

Abstract: The RNA helicase A (RHA) is a member of DExH-box helicases and characterized by two double-stranded RNA binding domains at the N-terminus. RHA unwinds double-stranded RNA in vitro and is involved in RNA metabolisms in the cell. RHA is also hijacked by a variety of RNA viruses to facilitate virus replication. Herein, this review will provide an overview of the role of RHA in the replication of RNA viruses.

Keywords: RNA helicase A; RNA virus; transcription; translation; replication

1. Introduction

RNA Helicase A (RHA), also known as DHX9 or Nuclear DNA Helicase II (NDH II), is a member of DExH-box family of superfamily 2 (SF2) helicases. RHA plays regulatory roles in a variety of cellular processes including transcription [1], translation [2], RNA splicing [3–5], RNA editing [1], RNA transport [6,7], microRNA genesis [8], circular RNA genesis [6,9–11], and maintenance of genomic stability [12,13].

RHA is predominantly retained in the nucleus, but able to shuttle back to the cytoplasm to fulfill its duty [14,15]. Human RHA was first isolated from nuclear extracts of HeLa cells [16]. RHA can unwind duplex RNA possessing a 3′ single-stranded RNA (ssRNA) tail [17] by hydrolyzing nucleoside triphosphates as energy resources [18]. The full-length RHA is a 140-kDa protein composed of 1270 amino acids [19]. RHA contains two double-stranded RNA-binding domains (dsRBDs) at its N-terminus and a helicase core domain in the middle region of the protein [19] (Figure 1). A helicase-associated domain 2 (HA2) is adjacent to the C-terminal end of the helicase core domain [20]. An oligonucleotide/oligosaccharide-binding fold (OB-fold) [21] and a glycine-rich RGG-box are located at the C-terminus of RHA [19]. In addition, RHA has a minimal transactivation domain (MTAD) located in between the second dsRBD (dsRBD2) and helicase core domain [22] and nuclear localization/export signals (NLS/NES) in between OB-fold and RGG-box [22].

RNA viruses have an RNA genome. RHA has been found to interact with the RNA or/and proteins of at least 10 RNA viruses from seven families including Arteriviridae, Flaviviridae, Hepeviridae, Orthomyxoviridae, Picornaviridae, Retroviridae, and Togaviridae. The involvement of RHA in the replication of a broad variety of RNA viruses may imply that it has potential to serve as an anti-viral target.
Figure 1. Schematic view of domains of RHA. The numbers represent the positions of RHA amino acid (aa) residues that delineate each functional domain or motif. dsRBD1: double-stranded RNA-binding domain 1; dsRBD2: double-stranded RNA-binding domain 2; MTAD: minimal transactivation domain; HA2: helicase-associated domain 2; OB-fold: oligonucleotide/oligosaccharide-binding fold; NLS/NES: nuclear localization/export signals.

2. RHA in Replication of RNA Viruses

2.1. Arteriviridae Family: Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

PRRSV is an enveloped positive-sense RNA virus that belongs to the family Arteriviridae [23]. The viral RNA genome is approximately 15 kb in length and contains at least 10 open reading frames (ORFs). The viral nonstructural protein Nsp9 is a core component of the viral replication and transcription complex (RTC) crucial for virus replication [24]. The nucleocapsid (N) protein is responsible for the packaging of viral genome [25].

N protein interacts with Nsp9 to facilitate the production of both viral RNA and infectious progeny viruses [26]. RHA associates with N protein [27,28]. This association can redistribute RHA from the nucleus into the cytoplasmic foci where RTC-mediated virus replication occurs [26]. In addition to viral genomic RNA (gRNA), the replication of PRRSV also produces a set of co-terminal subgenomic mRNAs (sgmRNAs) from which the viral structural proteins are translated [29]. Knockdown of RHA increases the ratio of short sgmRNAs [26]. On the other hand, overexpression of RHA increases the ratio of both longer sgmRNAs and the gRNA and enhances the production of progeny viruses [26]. These results imply that RHA is recruited by the N protein during virus replication to facilitate the synthesis of longer viral RNA [26].

2.2. Flaviviridae Family: Bovine Viral Diarrhea Virus (BVDV), Classical Swine Fever Virus (CSFV), Dengue Virus (DENV), and Hepatitis C Virus (HCV)

So far, RHA has been found to regulate the replication of at least four viruses from the family Flaviviridae.

2.2.1. BVDV

BVDV is a member of the Pestivirus genus of the family Flaviviridae [30]. BVDV genome is a positive-sense, single-stranded RNA of about 12.3 kb in length and contains one ORF that is flanked by untranslated regions (UTRs) at the 5' and 3' ends [31]. UV cross-linking/label transfer method identified that RHA specifically associates with both 3'- and 5'-UTRs. Further biochemical analysis reveals that RHA along with other cellular proteins including NF90/NFAR-1 and NF45 form a complex to establish a functional bridge between 3'- and 5'-UTRs. Depletion of RHA with small interfering RNA (siRNA) significantly reduces the replication of viral RNA [31].

2.2.2. CSFV

CSFV belongs to the Pestivirus genus of the family Flaviviridae. CSFV infection of pig causes swine fever [32].

The genome of CSFV is a positive-sense, single-stranded RNA that contains a single large ORF. The translation of ORF produces a polyprotein that will be further processed by cleavage into a series of smaller mature proteins including structural proteins (C, Erns, E1, E2) and nonstructural proteins (Npro, p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B) [30]. 5'-UTR and 3'-UTR flank the ORF and regulate both the protein translation and the replication of viral RNA [33].
Affinity chromatography and UV-crosslinking assays identified that RHA specifically binds to the 3'- and 5'-UTRs [34]. Depletion of RHA in PK-15 cells (porcine kidney cell line) significantly reduces the levels of viral RNA and NS3 protein, and the titers of progeny viruses. The results indicate that RHA is an important cellular factor involved in both the translation and the replication of CSFV [34].

2.2.3. DENV

Dengue virus is a mosquito-borne virus with four major serotypes (DENV1 to DENV4) [35]. Dengue virus infection causes dengue fever, dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS) [35]. DENV infection is a significant global health concern, particularly for persons living in tropical and subtropical areas. Approximately 100 million cases of dengue virus infection are reported globally per year [36].

The genome of DENV is a positive-sense, single-stranded RNA with 10.7 kb in length and encodes a single large polyprotein precursor of 3391 amino-acid residues [37]. Once the virion enters the host cell, the viral genome RNA is released into the cytoplasm to produce a large polyprotein precursor [37]. The polyprotein is processed by cellular and viral proteases into three structural proteins (C: capsid protein; prM/M: membrane protein; and E: envelope protein) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [35]. The NS proteins form a replicase complex in the perinuclear region of the endoplasmic reticulum (ER) to initiate viral RNA synthesis and protein translation [38].

DENV genome also contains short noncoding regions at both 5' and 3' ends [39]. Both 5'-m7GpppG cap and a conserved 3'-terminal stem loop (3'-SL) [40] are involved in the regulation of viral RNA translation and replication by interacting with cellular and/or viral proteins [41]. Recently, a variety of host factors have been identified to be involved in DENV replication [42–44]. RHA binds to DENV 3'-SL and forms a heterodimer with NF90. NF90 is a double-stranded RNA binding domain-containing protein that positively regulates dengue virus replication [45]. DENV infection partially redistributes RHA from the nucleus into the cytoplasm [46]. Knockdown of RHA in three permissive cell lines including A549, MDM (monocyte-derived macrophage), and HepG2 cells significantly reduces the replication of DENV strains NGC and 16681 and other flavivirus Japanese encephalitis virus (JEV), but slightly affects the replication of Zika virus (ZIKV). RHA does not affect the attaching of DENV virion to the cell, nor virus endocytosis [46]. RHA does not promote viral protein translation, but is required for DENV RNA replication by binding to viral RNA and associating with viral NS1, NS2B3, and NS4B but not NS5. Furthermore, the K417R mutant RHA lacking ATP binding activity works as efficiently as wild-type protein to facilitate virus replication, indicating that the ATPase/helicase activity of RHA is dispensable for the function of RHA in DENV replication [46].

2.2.4. HCV

HCV is the causative pathogen for hepatitis C and some human cancers such as hepatocellular carcinoma (HCC) and lymphomas [47]. HCV is a small enveloped RNA virus. HCV genome is a positive-sense, single-stranded RNA of about 9.7 kb in length and contains only one ORF flanked by UTRs at the 5'- and 3'-ends [48].

The 5'-UTR of HCV regulates both the translation and the replication of the viral RNA [48]. In a biotin-tagged RNA pulldown assay, RHA was found to associate with the cognate double-stranded RNA of 5'-UTR [49]. Depletion of RHA reduces both viral RNA synthesis and the levels of viral proteins including NS3 and NS5A, indicating that RHA is an essential cellular protein for HCV replication [50].
2.3. Hepeviridae Family: Hepatitis E Virus (HEV)

HEV belongs to the genus Orthohepevirus of the family Hepeviridae. It is the causative pathogen of Hepatitis E that is characterized by liver inflammation and usually follows an acute and self-limiting course of illness [51]. The viral particle is nonenveloped and contains a positive-sense, single-stranded RNA of about 7.2 kb in length. The viral genome contains three ORFs (ORF1, ORF2, and ORF3) that are flanked by UTRs at the 5’ and 3’ ends [52]. The UTRs of HEV genome function as cis-acting elements to control the initiation of virus replication [53].

RNA affinity chromatography followed by mass spectrometry analysis identified that RHA specifically interacts with the 3’-UTR of HEV in three mammalian cell lines (HepG2/C3A, A549, and Caco2), but the biological significance of these interactions is not known [54].

2.4. Orthomyxoviridae Family: Influenza A

Influenza A viruses cause acute respiratory disease in humans and a variety of animal species. The infection of human influenza A virus can cause seasonal epidemics of influenza [55]. Influenza A virus is an enveloped virus with a genome made up of eight negative sense, single-stranded, RNA segments, which encode 11 viral proteins [56].

Non-structural protein 1 (NS1) regulates virus replication, viral protein synthesis, host immune responses, and cellular signaling pathways [57]. NS1 complexes with RHA in an RNA-dependent manner during virus replication in A549 cells [58]. Depletion of RHA results in a significant reduction in virus yield, polymerase activity, and the levels of all species of viral RNAs including mRNA, cRNA, and vRNA during virus replication, indicating that RHA participates in both viral RNA transcription and replication. The ATPase-dependent helicase activity is required for the function of RHA in influenza A replication [58].

2.5. Picornaviridae Family: Foot and Mouth Disease Viruses (FMDV)

FMDV belongs to the Apthovirus genus of the family Picornaviridae [59]. FMDV infection causes a highly contagious disease for cloven-hoofed animals [60]. The viral particle is a nonenveloped icosahedron containing a positive-sense, single-stranded RNA genome. A single ORF in the RNA genome is flanked by UTRs at the 5’- and 3’-ends. The genomic RNA serves as not only the mRNA to produce a viral polyprotein but also the template for RNA synthesis [61].

FMDV replication redistributes RHA from the nucleus to the cytoplasm of infected bovine kidney LFBK cells by suppressing the methylation of RHA [62]. The cytoplasmic RHA associates with FMDV 2C and 3A as well as cellular poly(A) binding protein (PABP) and is in close proximity to the foci where the virus is replicating [62]. In vitro biochemical analysis reveals that RHA binds specifically to the S fragment of FMDV 5’-UTR, suggesting that it may interact with the virus genome in the host cell. The depletion of RHA by siRNA significantly reduces both the titer of progeny viruses and the expression of viral proteins including 3Dpol. These results indicate that RHA plays an essential role in the replication of FMDV probably by forming ribonucleoprotein complexes with viral and cellular proteins including 2C, 3A, and PABP at the 5’-UTR of viral genome [62].

2.6. Retroviridae Family: Human Immunodeficiency Virus Type 1 (HIV-1) and Other Retroviruses

HIV-1 is a member of lentivirus of the family Retroviridae. HIV-1 infection can cause the acquired immune deficiency syndrome (AIDS) [63]. Upon entry into host cells, the genomic RNA of HIV-1 is reversely transcribed into minus-strand strong-stop cDNA (-sscDNA) followed by conversion into double-stranded DNA that integrates into the host cell chromosomes as a provirus. The transcription of proviral DNA produces a full-length, unspliced viral RNA, which is translated into Gag and GagPol and later encapsidated into
the viral particles as viral RNA genome. tRNA\textsuperscript{\text{15y3}} is selectively packaged into viral particles and annealed to viral genomic RNA as a primer in reverse transcription [64].

RHA associates with HIV-1 Gag in an RNA-dependent manner and is incorporated into HIV-1 particles (Roy et al., 2006). HIV-1 particles produced from RHA-depleted 293T cells are less infectious and have a reduced ability to generate -sscDNA in newly infected cells. These phenomena are attributed to the reduced annealing of tRNA\textsuperscript{\text{15y3}} to the genomic viral RNA (Roy et al., 2006; Xing et al., 2011) and the compromised processivity of reverse transcription by reverse transcriptase (Brady et al., 2019).

The annealing of tRNA\textsuperscript{\text{15y3}} to viral genomic RNA is initiated by Gag [65] and then fine-tuned by nucleocapsid (NC) protein, a proteolytic derivative of Gag [66]. RHA promotes Gag-mediated annealing of tRNA\textsuperscript{\text{15y3}} by altering viral RNA conformation [67]. Enhancement of tRNA\textsuperscript{\text{15y3}} annealing plausibly benefits from the repetitive nature of unwinding by RHA, because the repetitive unwinding makes the unwound RNA strand more accessible for pairing with the third incoming RNA [17].

RHA promotes the transcription of HIV-1, facilitates the export of viral RNAs from the nucleus, and also affects the splicing process. The HIV-1 trans-activation response (TAR) element is a conserved RNA fragment that displays a dynamic hairpin structure [68]. The TAR element serves as the binding site for viral Tat protein and other cellular proteins including the cis-acting transactivation response element-binding protein (TRBP) in trans activation of HIV-1 long terminal repeat promoter [69]. The TAR element is also a preferred target of dsRBDS of RHA in both in vitro and in vivo assays [70]. Overexpression of wild-type RHA rather than mutant RHA with defect in ATP\textsubscript{ase} activity profoundly enhances viral mRNA synthesis and virion production [70]. RHA stimulates HIV-1 transcription by enhancing the occupancy of RNA polymerase II (RNAP II) on the proviral DNA [21]. The MTAD of RHA is critical to RHA-mediated activation of HIV-1 transcription, since deletions in MTAD significantly reduce the steady-state level of HIV-1 RNA transcripts [71].

In addition to full-length, unsliced (~ 9.2 kb) viral RNA, HIV-1 transcription also produces singly spliced (~ 4.0 kb) or multiply spliced (~ 1.8 kb) RNAs [72]. The singly spliced RNAs encode viral Env, Vpu, Vpr, or Vif proteins, whereas multiply spliced RNAs encode Tat, Rev, and Nef proteins. HIV-1 also produces a number of exon 6D-containing spliced viral RNAs encoding chimeric protein Tev (Tat–Env–Rev fusion protein) [73,74]. Overexpression of RHA increases the ratio of unsliced and singly spliced RNAs to multiply spliced ones [75]. Additionally, mutation analysis shows that OB-fold of RHA can modulate HIV-1 RNA splicing [21].

The nuclear export of unsliced or singly spliced HIV-1 RNAs is mediated by the interaction of trans-acting viral protein Rev with the cis-acting Rev response RNA element (RRE) [76]. RHA binds to HIV-1 RRE independently of Rev to promote nuclear export of viral RNAs in a helicase activity-dependent manner [75].

RHA promotes the translation of HIV-1 Gag by binding to the posttranscription control element (PCE) in the 5′-UTR of HIV-1 RNA [77]. The ATP\textsubscript{ase}-dependent helicase activity is required for the role of RHA in Gag translation. PCE is located within RU5 region of HIV-1 5′-UTR. PCE is also conserved in the genomes of other retroviruses including spleen necrosis virus (SNV) [78,79], reticuloendotheliosis virus A (REV-A), human T-cell leukemia virus type 1 (HTLV-1) [80], Mason–Pfizer monkey virus (MPMV) [81], or a set of cellular mRNAs [82]. Studies with HTLV-1 reveal that the interaction of RHA with PCE can promote the polysome association with viral RNA [80].

The interaction of RHA with HIV-1 was investigated by performing a genomewide analysis [83]. 5′-UTR of HIV-1 RNA is the major target for RHA, whereas RRE is the minor target during virus replication. Both dsRBDS1 and dsRBDS2 of RHA are essential for the binding of RHA to HIV-1 RNA, because deletion of either one leads to undetectable association of RHA with HIV-1 RNA in the cells [83]. Furthermore, the mutant RHA with deletion of either dsRBDS1 or dsRBDS2 does not promote the in vivo annealing of tRNA\textsuperscript{\text{15y3}} to viral RNA, nor is the mutant RHA packaged into viral particles [83]. Two conserved
lysine residues in each dsRBD of RHA, i.e., K54 and K55 in dsRBD1 and K235 and K236 in dsRBD2, are critical to the binding of RHA to HIV-1 RNA during virus replication [84].

2.7. Togaviridae Family: Chikungunya Virus (CHIKV)

CHIKV belongs to the genus Alphavirus of the family Togaviridae. The virus is transmitted by mosquitoes. CHIKV infection causes fever, rash, headache, joint and muscle pains, or joint swelling [85].

CHIKV genome is a positive-sense, single-stranded RNA of 11.6 kb in length [86]. It contains two ORFs, ORF1 and ORF2, that encode nonstructural and structural proteins, respectively [87]. Upon infection of host cell, the ORF1 is translated directly into P1234 and P123 polyproteins that will be further processed by self-cleavage to produce mature nonstructural proteins (nsP1, nsP2, nsP3, and nsP4) [87].

CHIKV infection of human HeLa cells significantly decreases the nuclear level of RHA in the cell and redeploys a fraction of RHA into plasma membrane-proximal cytoplasmic foci where the virus is replicating [88]. Immunoprecipitation experiments reveal that RHA complexes with CHIKV genomic RNA and nsPs in infected cells mainly by direct binding to the hypervariable domain (HVD) of nsP3. However, depletion of RHA with siRNA or clusters of regularly interspaced short palindromic repeats (CRISPR)/Cas9-mediated genome editing significantly enhances viral RNA synthesis. In contrast, RHA depletion dramatically decreases the expression of early nsPs, indicating that RHA is required for the translation of viral proteins. Taken together, RHA plays two distinct roles in CHIKV replication, i.e., inhibiting CHIKV RNA replication but enhancing the translation of the incoming viral genome [88].

3. Concluding Remarks

The growing evidences show that RHA associates with a broad variety of RNA viruses. In addition to serving as a proviral factor in RNA virus replication, RHA can act as an RNA sensor in the innate immune system. In myeloid dendritic cells (mDCs), RHA interacts with IPS-1 to sense double stranded RNA in the activation of nuclear factor kappa B (NF-kB) and IFN regulatory factor 3 [89]. Knockdown of RHA dramatically reduced the ability of mDCs to produce IFN-α/β and proinflammatory cytokines in response to infection of influenza A and reovirus [89]. Based on the available results, while the mechanisms by which RHA participates in the replication of different RNA viruses would be diverse, we still can see some characteristics that appear to be common. For example, studies reveal that the helicase activity is often required for the tested functions of RHA during virus replication and RHA always associates with the specific region of viral RNAs, such as 5' and/or 3' UTRs that usually adopt complexed secondary structures (Table 1). These features are reminiscent of the findings that RHA preferably binds to the double-stranded RNA rather than single-stranded one and is able to unwind the dsRNA [17,18]. Therefore, it would be plausible to postulate that RHA is employed to remodel the complexed RNA structure by using its helicase activity to regulate virus replication at diverse steps including transcription, translation, RNA synthesis, or tRNA<sup>5′</sup> annealing. However, how the RHA recognizes and then binds to the specific region in the viral RNA has yet to be explored. Several viral proteins have been shown to associate with or bind directly to the RHA during virus replication (Table 1). Those viral proteins may help to hijack RHA and then redirect the RHA to the places where it is needed. To understand the role of RHA in virus replication also requires the insightful information about the interactions of RHA with viral proteins or RNA elements.
Table 1. The role of RHA in the replication of RNA viruses. ?: not determined.

| Virus     | Family            | Biological Processes Affected | Helicase Activity Required (+) or not (-) | Viral Nucleic Acids Involved | Viral Protein Involved |
|-----------|-------------------|--------------------------------|------------------------------------------|-----------------------------|-----------------------|
| PRRSV     | Arteriviridae     | Genomic replication [26]      | ?                                        | ?                           | N                     |
| BVDV      | Flaviviridae      | Genomic replication [31]      | ?                                        | 3'-UTR, 5'-UTR              | ?                     |
| CSFV      | Flaviviridae      | Translation and genomic replication [34] | ?                                        | 3'-UTR, 5'-UTR              | ?                     |
| DENV      | Flaviviridae      | Genomic replication [46]      | -                                        | 3'-UTR                      | NS1, NS2B3, NS4B      |
| HCV       | Flaviviridae      | Translation and genomic replication [49,50] | ?                                        | 5'-UTR                      | ?                     |
| HEV       | Hepeviridae       | Unknown [54]                  | ?                                        | 3'-UTR                      | ?                     |
| Influenza A | Orthomyxoviridae | Transcription and genomic replication [58] | +                                        | ?                           | NS1                  |
| FMDV      | Picornaviridae    | Translation and genomic replication [62] | ?                                        | 5'-UTR                      | 2C and 3A             |
| HIV-1     | Retroviridae      | Transcription [70]            | +                                        |                              | Gag                  |
|           |                   | Translation [77]              | +                                        |                              |                       |
|           |                   | Reverse transcription [90]    | +                                        | 5'-UTR, RRE                 | Gag                  |
|           |                   | Splicing [21]                | +                                        |                              |                       |
|           |                   | RNA export [25]              | +                                        |                              |                       |
| CHIKV     | Togaviridae       | Translation and genomic replication [88] | ?                                        | ?                           | Nsp3                 |

**Abbreviations:** BVDV: bovine viral diarrhea virus; CHIKV: Chikungunya virus; CRISPR: clusters of regularly interspaced short palindromic repeats; CSFV: classical swine fever virus; DENV: Dengue virus; dsRBD: double-stranded RNA-binding domain; FMDV: foot and mouth disease viruses; gRNA: genomic RNA; HA2: helicase-associated domain 2; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HEV: hepatitis E virus; HIV-1: human immunodeficiency virus type 1; HTLV-1: human T-cell leukemia virus type 1; JEV: Japanese encephalitis virus; mDCs: myeloid dendritic cells; MTAD: minimal transactivation domain; NF-κB: nuclear factor kappa B; N protein: nucleocapsid protein; NC: nucleocapsid; NLS/NES: nuclear localization/export signal; NDH II: Nuclear DNA Helicase II; NS1: non-structural protein 1; OB-fold: oligonucleotide/oligosaccharide-binding fold; ORF: open reading frame; PABP: poly(A) binding protein; PRRSV: porcine reproductive and respiratory syndrome virus; RHA: RNA helicase A; RNAP II: RNA polymerase II; RRE: Rev response RNA element; RTC: replication and transcription complex; siRNA: small interfering RNA; SF2: superfamily 2; sgmRNA: subgenomic mRNA; -sscDNA: minus-strand strong-stop cDNA; ssRNA: single-stranded RNA; TAR: trans-activation response; Tev: Tat–Env–Rev fusion protein; UTR: untranslated region; WNV: West Nile virus; YFV: yellow fever virus; ZIKV: Zika virus.
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References

1. Hong, H.; An, O.; Chan, T.H.M.; Ng, V.H.E.; Kwok, H.S.; Lin, J.S.; Qi, L.; Han, J.; Tay, D.J.T.; Tang, S.J.; et al. Bidirectional regulation of adenosine-to-inosine (A-to-I) RNA editing by DEAH box helicase 9 (DHX9) in cancer. *Nucleic Acids Res.* 2018, 46, 7953–7969, doi:10.1093/nar/gky396.

2. Singh, G.; Fritz, S.E.; Seufzer, B.; Boris-Lawrie, K. The mRNA encoding the JUND tumor suppressor detains nuclear RNA-binding proteins to assemble polysomes that are unaffected by mTOR. *J. Biol. Chem.* 2020, 295, 7763–7773, doi:10.1074/jbc.RA120.012005.

3. Huan, W.; Zhang, J.; Li, Y.; Zhi, K. Involvement of DHX9/YB-1 complex induced alternative splicing of Krüppel-like factor 5 mRNA in phenotypic transformation of vascular smooth muscle cells. *Am. J. Physiol. Cell Physiol.* 2019, 317, C262–C269, doi:10.1152/ajpcell.00067.2019.

4. Chakraborty, P.; Huang, J.T.J.; Hiom, K. DHX9 helicase promotes R-loop formation in cells with impaired RNA splicing. *Nat. Commun.* 2018, 9, 1–14, doi:10.1038/s41467-018-06677-1.

5. Hu, Z.; Dong, L.; Li, S.; Li, Z.; Qiao, Y.; Li, Y.; Ding, J.; Chen, Z.; Wu, Y.; Wang, Z.; et al. Splicing Regulator p54nrb/Non–POU Domain–Containing Octamer-Binding Protein Enhances Carcinogenesis Through Oncogenic Isoform Switch of MYC Box-Dependent Interacting Protein 1 in Hepatocellular Carcinoma. *Hepatology* 2020, 72, 548–568, doi:10.1002/hep.31062.

6. Aktaş, T.; Ilk İbrahim, A.; Maticzka, D.; Bhardwaj, V.; Rodrigues, C.P.; Mittler, G.; Manke, T.; Backofen, R.; Akhtar, A. DHX9 suppresses RNA processing defects originating from the Alu invasion of the human genome. *Nat. Cell Biol.* 2017, 544, 115–119, doi:10.1038/nature21715.

7. Kim, J.-E.; Hong, Y.H.; Kim, J.Y.; Jeon, G.S.; Jung, J.H.; Yoon, B.-N.; Son, S.-Y.; Lee, K.-W.; Kim, J.-I.; Sung, J.-J. Altered nucleocytoplasmic proteome and transcriptome distributions in an in vitro model of amyotrophic lateral sclerosis. *PLoS ONE* 2017, 12, e0176462, doi:10.1371/journal.pone.0176462.

8. Kawai, S.; Amano, A. BRCA1 regulates microRNA biogenesis via the DROSHA microprocessor complex. *J. Cell Biol.* 2012, 197, 201–208, doi:10.1083/jcb.201110008.

9. Zhou, J.-Z.; Hu, M.-R.; Diao, H.-L.; Wang, Q.-W.; Huang, Q.; Ge, B.-J. Comprehensive analysis of differentially expressed circRNAs revealed a ceRNA network in pancreatic ductal adenocarcinoma. *Arch. Med. Sci.* 2019, 15, 979–991, doi:10.5114/ams.2019.85204.

10. Sekiba, K.; Otsuka, M.; Ohno, M.; Kishikawa, T.; Yamagami, M.; Suzuki, T.; Ishibashi, R.; Seimiya, T.; Tanaka, E.; Koike, K. DHX9 regulates production of hepatitis B virus-derived circular RNA and viral protein levels. *Oncotarget* 2018, 9, 20953–20964, doi:10.18632/oncotarget.25104.

11. Ottesen, E.W.; Luo, D.; Seo, J.; Singh, N.N.; Singh, R.N. HumanSurvival Motor Neuron genes generate a vast repertoire of circular RNAs. *Nucleic Acids Res.* 2019, 47, 2884–2905, doi:10.1093/nar/gkz034.

12. Jain, A.; Bacolla, A.; Del Mundo, I.M.; Zhao, J.; Wang, G.; Vasquez, K.M. DHX9 helicase is involved in preventing genomic instability induced by alternatively structured DNA in human cells. *Nucleic Acids Res.* 2013, 41, 10345–10357, doi:10.1093/nar/gkt804.

13. Scott, D.D.; Trahan, C.; Zindy, P.J.; Aguilar, L.C.; Delubac, M.Y.; Van Nostrand, E.L.; Adivarahan, S.; Wei, K.E.; Yeo, G.W.; Zenklusen, D.; et al. Nol12 is a multifunctional RNA binding protein at the nexus of RNA and DNA metabolism. *Nucleic Acids Res.* 2017, 45, 12509–12528, doi:10.1093/nar/gkt963.

14. Zhang, S.; Maacke, H.; Grosse, F. Molecular Cloning of the Gene Encoding Nuclear DNA Helicase II. A bovine homologue of human RNA helicase A and drosophila Mle protein. *J. Biol. Chem.* 1995, 270, 16422–16427, doi:10.1074/jbc.270.27.16422.

15. Tang, H.; McDonald, D.; Middlesworth, T.; Hope, T.J.; Wong-Staal, F. The Carboxyl Terminus of RNA Helicase A Contains a Bidirectional Nuclear Transport Domain. *Mol. Cell. Biol.* 1999, 19, 3540–3550, doi:10.1128/mcb.19.5.3540.

16. Lee, C.; Hurwitz, J. A new RNA helicase isolated from HeLa cells that catalytically translocates in the 3’ to 5’ direction. *J. Biol. Chem.* 1992, 267, 4398–4407, doi:10.1016/S0021-9258(18)42849-9.

17. Koh, H.R.; Xing, L.; Kleiman, L.; Myong, S. Repetitive RNA unwinding by RNA helicase A facilitates RNA annealing. *Nucleic Acids Res.* 2014, 42, 8556–8564, doi:10.1093/nar/gku523.

18. Lee, C.; Hurwitz, J. Human RNA helicase A is homologous to the maleless protein of Drosophila. *J. Biol. Chem.* 1993, 268, 16822–16830, doi:10.1016/s0021-9258(19)85490-x.
19. Zhang, S.; Grosse, F. Domain Structure of Human Nuclear DNA Helicase II (RNA Helicase A). *J. Biol. Chem.* 1997, 272, 11487–11494, doi:10.1074/jbc.272.17.11487.

20. Xing, L.; Zhao, X.; Niu, M.; Kleiman, L. Helicase associated 2 domain is essential for helicase activity of RNA helicase A. *Biochim. Biophys. Acta (BBA) Proteins Proteom.* 2014, 1844, 1757–1764, doi:10.1016/j.bbapap.2014.07.001.

21. Xing, L.; Niu, M.; Kleiman, L. Role of the OB-fold of RNA helicase A in the synthesis of HIV-1 RNA. *Biochim. Biophys. Acta (BBA) Bioenerg.* 2014, 1839, 1069–1078, doi:10.1016/j.bbgen.2014.08.008.

22. Aratani, S.; Oishi, T.; Fujita, H.; Nakazawa, M.; Fujii, R.; Immoto, N.; Yoneda, Y.; Fukamizu, A.; Nakajima, T. The nuclear import of RNA helicase A is mediated by importin-a3. *Biochem. Biophys. Res. Commun.* 2006, 340, 125–133, doi:10.1016/j.bbrc.2005.11.161.

23. Cavanagh, D. Nidovirales: A new order comprising Coronaviridae and Arteriviridae. *Arch. Virol.* 1997, 142, 629–633.

24. Knoops, K.; Bárca, M.; Limpenz, R.W.A.L.; Koster, A.J.; Mommaas, A.M.; Snijder, E.J. Ultrastructural Characterization of Arterivirus Replication Structures: Reshaping the Endoplasmic Reticulum To Accommodate Viral RNA Synthesis. *J. Virol.* 2011, 86, 2474–2487, doi:10.1128/jvi.06677-11.

25. Yoo, D.; Wootton, S.K.; Li, G.; Song, C.; Rowland, R.R. Colocalization and Interaction of the Porcine Arterivirus Nucleocapsid Protein with the Small Nucleolar RNA-Associated Protein Fibrillarin. *J. Virol.* 2003, 77, 12173–12183, doi:10.1128/77.22.12173-12183.2003.

26. Liu, L.; Tian, J.; Nan, H.; Tian, M.; Li, Y.; Xu, X.; Huang, B.; Zhou, E.; Hiscox, J.A.; Baicheng, H. Porcine Reproductive and Respiratory Syndrome Virus Nucleocapsid Protein Interacts with Nsp9 and Cellular DHX9 To Regulate Viral RNA Synthesis. *J. Virol.* 2016, 90, 5384–5398, doi:10.1128/jvi.03216-15.

27. Jourdan, S.S.; Osorio, F.; Hiscox, J.A. An interactome map of the nucleocapsid protein from a highly pathogenic North American porcine reproductive and respiratory syndrome virus strain generated using SILAC-based quantitative proteomics. *Proteomics* 2012, 12, 1015–1023, doi:10.1002/pmic.201100469.

28. Liu, L.; Lear, Z.; Hughes, D.J.; Wu, W.; Zhou, E.-M.; Whitehouse, A.; Chen, H.; Hiscox, J.A. Resolution of the cellular proteome of the nucleocapsid protein from a highly pathogenic isolate of porcine reproductive and respiratory syndrome virus identifies PARP-1 as a cellular target whose interaction is critical for virus biology. *Vet. Microbiol.* 2015, 176, 109–119, doi:10.1016/0303.11.023.

29. Kappes, M.A.; Faaberg, K.S. PRRSV structure, replication and recombination: Origin of phenotype and genotype diversity. *Virology* 2015, 479, 475–486, doi:10.1016/j.virol.2015.02.012.

30. Tautz, N.; Tews, B.A.; Meyers, G. The Molecular Biology of Pestiviruses. *Adv. Virus Res.* 2015, 93, 47–160, doi:10.1016/bs.avir.2015.03.002.

31. Isken, O.; Grassmann, C.W.; Sarisky, R.T.; Kann, M.; Zhang, S.; Grosse, F.; Kao, P.N.; Behrens, S. Members of the NF90/NFAR protein group are involved in the life cycle of a positive-strand RNA virus. *EMBO J.* 2003, 22, 5655–5665, doi:10.1093/emboj/cdg562.

32. Moenig, V.; Floegel-Niesmann, G.; Greiser-Wilke, I. Clinical Signs and Epidemiology of Classical Swine Fever: A Review of New Knowledge. *Vet. J.* 2003, 165, 11–20, doi:10.1016/s1090-2333(02)00112-0.

33. Fletcher, S.P.; Jackson, R.J. Pestivirus Internal Ribosome Entry Site (IRES) Structure and Function: Elements in the 5′ Untranslated Region Important for IRES Function. *J. Virol.* 2002, 76, 5024–5033, doi:10.1128/jvi.76.5024-5033.2002.

34. Sheng, C.; Yao, Y.; Chen, B.; Wang, Y.; Chen, J.; Xiao, M. RNA helicase is involved in the expression and replication of classical swine fever virus and interacts with untranslated region. *Virus Res.* 2013, 171, 257–261, doi:10.1016/j.virusres.2012.11.014.

35. Dwivedi, V.D.; Tripathi, I.P.; Tripathi, R.C.; Bharadwaj, S.; Mishra, S.K. Genomics, proteomics and evolution of dengue virus. *Briefings Func. Genom.* 2017, 16, 217–227, doi:10.1093/bfgp/elw040.

36. Messina, J.P.; Brady, O.J.; Scott, T.W.; Zou, C.; Pigott, D.M.; Duda, K.A.; Bhatt, S.; Katzelnick, L.; Howes, R.E.; Battle, K.E.; et al. Global spread of dengue virus types: Mapping the 70 year history. *Trends Microbiol.* 2014, 22, 138–146, doi:10.1016/j.tim.2013.12.011.

37. Cruz-Oliveira, C.; Freire, J.M.; Conceição, T.M.; Higa, L.M.; Castanho, M.A.; Da Poian, A.T. Receptors and routes of dengue virus entry into the host cells. *FEMS Microbiol. Rev.* 2015, 39, 155–170, doi:10.1093/femsre/fuu004.

38. Brand, C.; Bisaillon, M.; Geiss, B.J. Organization of the Flavivirus RNA replicase complex. *Wiley Interdiscip. Rev. RNA* 2017, 8, e1437, doi:10.1002/wrna.e1437.

39. Rodenhuis-Zybert, I.A.; Wilschut, J.; Smit, J.M. Dengue virus life cycle: Viral and host factors modulating infectivity. *Cell. Mol. Life Sci.* 2010, 67, 2773–2786, doi:10.1007/s00018-010-0357-z.

40. Brinton, M.A.; Fernandez, A.V.; Disposto, J.H. The 3′-nucleotides of flavivirus genomic RNA form a conserved secondary structure. *Virology* 1986, 153, 113–121, doi:10.1016/0042-6822(86)90012-7.

41. Holden, K.L.; Harris, E. Enhancement of dengue virus translation: Role of the 3′ untranslated region and the terminal 3′ stem-loop domain. *Virology* 2004, 329, 119–133, doi:10.1016/j.virol.2004.08.004.

42. Campos, R.K.; Wong, B.; Xie, X.; Lu, Y.-F.; Shi, P.-Y.; Pompon, J.; Garcia-Blanco, M.A.; Bradrick, S.S. RPLP1 and RPLP2 Are Essential Flavivirus Host Factors That Promote Early Viral Protein Accumulation. *J. Virol.* 2017, 91, 91, doi:10.1128/jvi.01706-16.

43. Lin, D.L.; Cherepanova, N.A.; Bozzacco, L.; Macdonald, M.R.; Gilmore, R.; Tai, A.W. Dengue Virus Hijacks a Noncanonical Oxidoreductase Function of a Cellular Oligosaccharidyltransferase Complex. *mBio* 2017, 8, e00939-17, doi:10.1128/mbio.00939-17.

44. Neufeldt, C.J.; Cortese, M.; Acosta, E.G.; Bartenschlager, R. Rewiring cellular networks by members of the Flaviviridae family. *Nat. Rev. Genet.* 2018, 16, 125–142, doi:10.1038/nrg.2017.170.
45. Gomila, R.C.; Martin, G.W.; Gehrke, L. NF90 Binds the Dengue Virus RNA 3’ Terminus and Is a Positive Regulator of Dengue Virus Replication. *PLoS ONE* **2011**, *6*, e16687, doi:10.1371/journal.pone.0016687.

46. Wang, Y.; Chen, X.; Xie, J.; Zhou, S.; Huang, Y.; Li, Y.-P.; Li, X.; Liu, C.; He, J.; Zhang, P. RNA Helicase A Is an Important Host Factor Involved in Dengue Virus Replication. *J. Virol.* **2018**, *93*, 93, doi:10.1128/jvi.01306-18.

47. Rusyn, I.; Lemon, S.M. Mechanisms of HCV-induced liver cancer: What did we learn from in vitro and animal studies? *Cancer Lett.* **2014**, *345*, 210–215, doi:10.1016/j.canlet.2013.06.028.

48. Kato, N. Genome of Human Hepatitis C Virus (HCV): Gene Organization, Sequence Diversity, and Variation. *Microb. Comp. Genom.* **2000**, *5*, 129–151, doi:10.1016/S0953-5230(00)00106-2.

49. Williams, B.; Masaki, T.; Shimakami, T.; Lemon, S.M. hnRNP L and NF90 Interact with Hepatitis C Virus 5’-Terminal Untranslated RNA and Promote Efficient Replication. *J. Virol.* **2014**, *88*, 7199–7209, doi:10.1128/jvi.00225-14.

50. He, Q.S.; Tang, H.; Zhang, J.; Truong, K.; Wong-Staal, F.; Zhou, D. Comparisons of RNAi approaches for validation of human RNA helicase A as an essential factor in hepatitis C virus replication. *J. Virol. Methods* **2008**, *154*, 216–219, doi:10.1016/j.jviromet.2008.08.005.

51. Khuroo, M.S. Discovery of hepatitis E: The epidemic non-A, non-B hepatitis 30 years down the memory lane. *Virus Res.* **2011**, *161*, 3–14, doi:10.1016/j.virusres.2011.02.007.

52. Haqshenas, G.; Meng, X.J. Determination of the nucleotide sequences at the extreme 5’ and 3’ ends of swine hepatitis E virus genome. *Arch. Virol.* **2001**, *146*, 2461–2467, doi:10.1007/s007050170016.

53. Graff, J.; Nguyen, H.; Kasornlekar, C.; Halbur, P.G.; Claire, M.S.; Purcell, R.H.; Emerson, S.U. In Vitro and In Vivo Mutational Analysis of the 3’-Terminal Regions of Hepatitis E Virus Genomes and Replicons. *J. Virol.* **2005**, *79*, 1017–1026, doi:10.1128/jvi.79.2.1017-1026.2005.

54. Paingankar, M.S.; Arunkalle, V.A. Identification and characterization of cellular proteins interacting with Hepatitis E virus untranslated regions. *Virus Res.* **2015**, *208*, 98–109, doi:10.1016/j.virusres.2015.06.006.

55. Neumann, G.; Wakaoaka, Y. Transmission of influenza A viruses. *Virology* **2015**, 234–246, doi:10.1016/j.viro.2015.03.009.

56. Samji, T. Influenza A: Understanding the Viral Life Cycle. *J. Biol. Med.* **2009**, *82*, 153–159.

57. Hale, B.G.; Randall, R.E.; Ortín, J.; Jackson, D. The multifunctional NS1 protein of influenza A viruses. *J. Gen. Virol.* **2008**, *89*, 2359–2376, doi:10.1099/vir.0.00460-0.

58. Lin, L.; Li, Y.; Pyo, H.-M.; Lu, X.; Raman, S.N.T.; Liu, Q.; Brown, E.G.; Zhou, Y. Identification of RNA Helicase A as a Cellular Factor That Interacts with Influenza A Virus NS1 Protein and Its Role in the Viral Life Cycle. *J. Virol.* **2011**, *86*, 1942–1954, doi:10.1128/jvi.00632-11.

59. Sáiz, M.; Núñez, J.J.; Jiménez-Clavero, M.A.; Baranowski, E.; Sobrino, F. Foot-and-mouth disease virus: Biology and prospects for disease control. *Microbes Infect.* **2002**, *4*, 1183–1192, doi:10.1016/s1286-4579(02)01644-1.

60. Grubman, M.J.; Baxt, B. Foot-and-Mouth Disease. *Clin. Microbiol. Rev.* **2004**, *17*, 465–493, doi:10.1128/cmr.17.2.465-493.2004.

61. Wimmer, E.; Kuhn, R.J.; Pincus, S.; Yang, C.-F.; Toyoda, H.; Nicklin, M.J.H.; Takeda, N. Molecular Events Leading to Picornavirus Genome Replication. *J. Cell Sci.* **1987**, *1987*, 251–276, doi:10.1242/jcs.1987.supplement_7.18.

62. Lawrence, P.; Rieder, E. Identification of RNA Helicase A as a New Host Factor in the Replication Cycle of Foot-and-Mouth Disease Virus. *J. Virol.* **2009**, *83*, 11356–11366, doi:10.1128/jvi.02677-08.

63. Sepkowitz, K.A. AIDS—The First 20 Years. *N. Engl. J. Med.* **2001**, *344*, 1764–1772, doi:10.1056/nejm200106073442306.

64. Kleiman, L.; Halwani, R.; Javanbakht, H. The selective packaging and annealing of primer tRNALys3 in HIV-1. *Curr. HIV Res.* **2004**, *2*, 163–175, doi:10.2174/1570162034384988.

65. Cen, S.; Huang, Y.; Khochrid, A.; Darlix, J.-L.; Wainberg, M.A.; Kleiman, L. The Role of Pr55gag in the Annealing of tRNA3Lys to Human Immunodeficiency Virus Type 1 Genomic RNA. *J. Virol.* **1999**, *73*, 4485–4488, doi:10.1128/jvi.73.9.4485-4488.1999.

66. Guo, F.; Saadatmand, J.; Niu, M.; Kleiman, L. Roles of Gag and NCp7 in facilitating Trna (Lys)(3) annealing to viral RNA in human immunodeficiency virus type 1. *J. Virol.* **2009**, *83*, 8099–8107, doi:10.1128/jvi.00488-09.

67. Xing, L.; Liang, C.; Kleiman, L. Coordinate Roles of Gag and RNA Helicase A in Promoting the Annealing of Formula to HIV-1 RNA. *J. Virol.* **2010**, *85*, 1847–1860, doi:10.1128/jvi.0210-10.

68. Lu, J.; Kadakkuzha, B.M.; Zhao, L.; Fan, M.; Qi, X.; Xia, T. Dynamic Ensemble View of the Conformational Landscape of HIV-1 TAR RNA and Allosteric Recognition. *Biochemistry* **2011**, *50*, 5042–5057, doi:10.1021/bi200495d.

69. Kulinski, T.; Olejniczak, M.; Huthoff, H.; Bielecki, L.; Pachul ska-Wieczorek, K.; Das, A.T.; Berkhour, B.; Adamiak, R.W. The Apical Loop of the HIV-1 TAR RNA Hairpin Is Stabilized by a Cross-loop Base Pair. *J. Biol. Chem.* **2003**, *278*, 38892–38901, doi:10.1074/jbc.m301939200.

70. Fuji, R.; Okamoto, M.; Aratani, S.; Oishi, T.; Ohshima, T.; Taira, K.; Baba, M.; Fukumizu, A.; Nakajima, T. A Role of RNA Helicase A in cis-Acting Transactivation Response Element-mediated Transcriptional Regulation of Human Immunodeficiency Virus Type 1. *J. Biol. Chem.* **2001**, *276*, 5445–5451, doi:10.1074/jbc.m006892200.

71. Xing, L.; Niu, M.; Zhao, X.; Kleiman, L. Roles of the Linker Region of Helicase A in HIV-1 RNA Metabolism. *PLoS ONE* **2013**, *8*, e78596, doi:10.1371/journal.pone.0078596.

72. Purcell, D.F.; Martin, M.A. Alternative splicing of human immunodeficiency virus type 1 mRNA regulates viral protein expression, replication, and infectivity. *J. Virol.* **1993**, *67*, 3635–3678, doi:10.1128/jvi.67.11.3635-3678.1993.

73. Benko, D.M.; Schwartz, S.; Pavlakis, G.N.; Felber, B.K. A novel human immunodeficiency virus type 1 protein, tev, shares sequences with tat, env, and rev proteins. *J. Virol.* **1990**, *64*, 2505–2518, doi:10.1128/jvi.64.6.2505-2518.1990.
Viruses 2021, 13, 361

74. Salfeld, J.; Göttlinger, H.; Sia, R.; Park, R.; Sodroski, J.; Haseltine, W. A tripartite HIV-1 tat-env-rev fusion protein. *EMBO J.* **1990**, 9, 965–970, doi:10.1002/j.1460-2075.1990.tb08195.x.

75. Li, J.; Tang, H.; Mullen, T.-M.; Westberg, C.; Reddy, T.R.; Rose, D.W.; Wong-Staal, F. A role for RNA helicase A in post-transcriptional regulation of HIV type 1. *Proc. Natl. Acad. Sci. USA* **1999**, 96, 709–714, doi:10.1073/pnas.96.2.709.

76. Fischer, U.; Huber, J.; Boelens, W.C.; Mattaj, I.W.; Lührmann, R. The HIV-1 Rev Activation Domain is a nuclear export signal that accesses an export pathway used by specific cellular RNAs. *Cell* **1995**, 82, 475–483, doi:10.1016/0092-8674(95)90436-0.

77. Boltinger, C.; Sharma, A.; Singh, D.; Yu, L.; Boris-Lawrie, K. RNA helicase A modulates translation of HIV-1 and infectivity of progeny virions. *Nucleic Acids Res.* **2010**, 38, 1686–1696, doi:10.1093/nar/gkp1075.

78. Butsch, M.; Hull, S.; Wang, Y.; Roberts, T.M.; Boris-Lawrie, K. The 5′ RNA Terminator of Spleen Necrosis Virus Contains a Novel Posttranscriptional Control Element That Facilitates Human Immunodeficiency Virus Rev/RE-Independent Gag Production. *J. Virol.* **1999**, 73, 4847–4855, doi:10.1128/jvi.73.6.4847-4855.1999.

79. Roberts, T.M.; Boris-Lawrie, K. The 5′ RNA Terminator of Spleen Necrosis Virus Stimulates Translation of Nonviral mRNA. *J. Virol.* **2000**, 74, 10229–10235, doi:10.1128/jvi.74.17.1111-1118.2000.

80. Boltinger, C.; Yilmaz, A.; Hartman, T.R.; Kovacic, M.B.; Fernandez, S.; Ye, J.; Forget, M.; Green, P.L.; Boris-Lawrie, K. RNA helicase A interacts with divergent lymphotropic retroviruses and promotes translation of human T-cell leukemia virus type 1. *Nucleic Acids Res.* **2007**, 35, 2629–2642, doi:10.1093/nar/gkm124.

81. Hull, S.; Boris-Lawrie, K. RU5 of Mason-Pfizer Monkey Virus 5′ Long Terminal Repeat Enhances Cytoplasmic Expression of Human Immunodeficiency Virus Type 1 gag-pol and Nonviral Reporter mRNA. *J. Virol.* **2002**, 76, 10211–10218, doi:10.1128/jvi.76.20.10211-10218.2002.

82. Hartman, T.R.; Qian, S.; Boltinger, C.; Fernandez, S.; Schoenberg, D.R.; Boris-Lawrie, K. RNA helicase A is necessary for translation of selected messenger RNAs. *Nat. Struct. Mol. Biol.* **2006**, 13, 509–516, doi:10.1038/nsmb1092.

83. Xing, L.; Niu, M.; Kleiman, L. In Vitro and In Vivo Analysis of the Interaction between RNA Helicase A and HIV-1 RNA. *J. Virol.* **2012**, 86, 13272–13280, doi:10.1128/jvi.01993-12.

84. Xing, L.; Niu, M.; Zhao, X.; Kleiman, L. Different activities of the conserved lysine residues in the double-stranded RNA binding domains of RNA helicase A in vitro and in the cell. *Biochim. Biophys. Acta (BBA) Gen. Subj.* **2014**, 1840, 2234–2243, doi:10.1016/j.bbagen.2014.04.003.

85. Busch, M.; Erickson, G. An overview of Chikungunya virus. *J. Am. Acad. Physician Assist.* **2015**, 28, 54–57, doi:10.1097/01.jaa.0000470441.99693.e1.

86. Kendall, C.; Khalid, H.; Müller, M.; Banda, D.H.; Kohl, A.; Merits, A.; Stonehouse, N.J.; Tuplin, A. Structural and phenotypic analysis of Chikungunya virus RNA replication elements. *Nucleic Acids Res.* **2019**, 47, 9296–9312, doi:10.1093/nar/gkz640.

87. Rupp, J.C.; Sokoloski, K.J.; Gebhart, N.N.; Hardy, R.W. Alphavirus RNA synthesis and non-structural protein functions. *J. Gen. Virol.* **2015**, 96, 2483–2500, doi:10.1099/jgv.0.000249.

88. Matkovic, R.; Bernard, E.; Fontanel, S.; Eldin, P.; Chazal, N.; Hersi, D.H.; Merits, A.; Pélaponèse, J.-M.; Briant, L. The Host DHX9 DExH-Box Helicase Is Recruited to Chikungunya Virus Replication Complexes for Optimal Genomic RNA Translation. *J. Virol.* **2018**, 92, doi:10.1128/jvi.01764–18.

89. Zhang, Z.; Yuan, B.; Lu, N.; Facchini, V.; Liu, Y.-J. DHX9 Pairs with IPS-1 To Sense Double-Stranded RNA in Myeloid Dendritic Cells. *J. Immunol.* **2011**, 187, 4501–4508, doi:10.4049/jimmunol.1101307.

90. Roy, B.B.; Hu, J.; Guo, X.; Russell, R.S.; Guo, F.; Kleiman, L.; Liang, C. Association of RNA Helicase A with Human Immunodeficiency Virus Type 1 Particles. *J. Biol. Chem.* **2006**, 281, 12625–12635, doi:10.1074/jbc.m510596200.