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Review

Virus infection-induced host mRNA degradation and potential application of live cell imaging

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Abstract

Viruses exist wherever there is life. They can cause allergy, immune response, inflammation, and even fatal diseases directly or indirectly. Accumulating evidence shows that host RNA undergoes rapid degradation during virus infection. Herein, we focus on several possible mechanisms of infection-induced host RNA turnover, which seems to be a common strategy for both prokaryotic and eukaryotic viruses during the very early stage of infection and a potential application of live cell imaging on its visualization.

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1. Introduction

When a virus/bacteriophage infects a host cell, it utilizes its host's molecular machinery to replicate, efficiently generating more than hundredfold of its progeny. In particular, the virus relies on the ribosomes in the host cell to translate viral messenger RNA (mRNA) into polypeptides. Many viruses also impair the translation of cellular mRNA [1–3], one of the mechanisms during the shift of gene expression from host to virus, a process termed “host shutoff”, in order to prevent the production of anti-viral, host protecting proteins [4].

Both eukaryotic and prokaryotic viruses are reported to have numerous mechanisms during ‘host shutoff’ that are either essential or can facilitate their propagation under changing

environment, such as host interferon antagonist, host DNA damage, host transcriptional and translational apparatuses modification [5–7]. Among them, virus infection-induced host RNA degradation has not been paid enough attention to, which might be the very early step after viral infection in most cases. Strict control of their gene expression regulates the timing of host factor suppression to maximize their replication.

2. Dominant host mRNA decay pathways

mRNA decay occurs in various pathways and is highly regulated in both eukaryotes and prokaryotes. Here we will mainly summarize the common pathways.

In mammalian cells, a majority of mRNAs are capped by a 5' 7-methyl-guanosine (m⁷G) and tailed by poly adenine (A) at 3' end. These features physically protect the mRNA ends from exonucleolytic decay that remove nucleotides from 5'-end (Xrn1 mainly in the cytoplasm and Xrn2 in the nucleus) or 3'-end (the exosome complex) [8]. These structures of mRNA also serve to recruit translation initiation machinery [9].

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Cellular deadenylase enzyme complexes, CCR4-NOT, PAN2-PAN3, and PARN are in charge of the deadenylation, which is the first and often the rate-limiting step of mRNA decay, resulting in the shortening of the poly (A) tail. Decapping enzymes are required in some cases for their mRNA turnover such as Dcp1, Dcp2, etc. [10].

In bacterial cells, the 3'-end and 5'-end of mRNA are likewise protected from exonucleolytic decay. mRNAs are 5'-end triphosphated instead of a 5' cap and transcriptionally terminated at 3'-end with stem-loop [11], which is sometimes followed by a poly (U) or poly (A) cluster. Endonucleolytic cleavage via endoribonuclease is one of the basal pathways for bacterial mRNAs such as that in *Escherichia coli* (*E. coli*) endoribonuclease E plays an essential role in its mRNA turnover process [12]. Another common route for bacteria mRNA decay is that a removal of two of the triphosphates via RNA 5' Pyrophosphohydrolase (RppH) [13] leaving a 5' monophosphate, causes the message to be destroyed by the exonuclease RNase J, which degrades 5' to 3', such as the mRNA turnover in *Bacillus subtilis* [11].

3. Virus/bacteriophage induces host RNA degradation

Promoting global mRNA degradation is reported to be one of the host shutoff mechanisms to block host gene expression among at least three different viral subfamilies, alpha-herpesviruses, gammaherpesviruses and betacoronaviruses [4,5]. Moreover, Gaglia et al.'s work showed that viral encoded proteins trigger host mRNA degradation by a primary endonucleolytic cleavage causing shutoff of host gene expression and a host exonuclease such as Xrn1, an important 5' to 3' exonuclease in human cells, were required in subsequent completion of host mRNA turnover [5]. As shown in Fig. 1A and B, nearly all viruses known drive widespread mRNA degradation triggered by either internal endonucleolytic cleavage or removal of 5' cap structure of the mRNA.

One typical example of alphaherpesviruses is herpes simplex virus 1 (HSV-1). Virion host shutoff protein (Vhs, previously UL41) having an RNase activity [14,15], is packaged in virions and released after infection [16,17]. Vhs is directed to mRNAs through interactions with the translation initiation factors eIF4H and eIF4AII [18] and thus induces host mRNA decline.

Several examples of gammaherpesviruses were also characterized: SOX in Kaposi's sarcoma-associated herpesvirus (KSHV) [19] is the homolog of the alkaline exonuclease of other herpesviruses, which has been shown to function as a DNase involved in processing and packaging the viral genome [20] as an endonuclease. During KSHV infection, SOX initiates host mRNA degradation by its endonucleolytic cleavage followed by rapid exonucleolytic degradation of host Xrn1 [21]. Recently, Mendez et al. demonstrated that SOX cleaves its target mRNA without other factors by *in vitro* cleavage assay [22]. Moreover, Muller et al. revealed that some mRNAs that 'overridden' SOX were also degraded, suggesting multiple viral endonucleases' existence [23].

BGLF5 in Epstein-Barr virus (EBV) plays a role in processing non-linear or branched viral DNA intermediates in

order to promote the production of mature packaged unit-length linear progeny viral DNA molecules [24], serves as an endoribonuclease of host mRNA after infection [25]. Similar to BGLF5, muSOX in murine herpesvirus 68 (MHV68), which is also alkaline exonuclease homolog, a member of the PD(D/E)XK restriction endonuclease superfamily have been demonstrated to induce host mRNA decay after infection via endonucleolytic attack [26].

Nsp1, a protein with no known similarity to cellular or viral nucleases from severe acute respiratory syndrome (SARS) coronavirus (SCoV) belonging to betacoronavirus family, is reported to induce host mRNA degradation by binding to 40S ribosome [27,28]. Nsp1 also prevents Sendai virus-induced endogenous IFN- β mRNA accumulation. Later Kamitani et al. found that Nsp1 bound to host 40S ribosomal subunit and inactivated the translational activity of the 40S subunits. Meanwhile, Nsp1-40S ribosome complex induces the modification of the 5' region of capped mRNA template and renders the template RNA translationally incompetent [1].

RNA endoribonuclease designated as PA-X, which is encoded in the genome of Influenza A virus (IAV), stimulates the decay of cellular mRNA [29]. Host RNA degradation was also reported in IAV-infected cells, though the sensitivity of host mRNAs to IAV-induced host shutoff varies. For example, host proteins that facilitate viral propagation (such as the proteins that maintain oxidative phosphorylation) are less attacked [4,30]. This suggests that like IAV, virus infection in fact effectively and strictly control the total pool of mRNAs in virus-infected cells, allowing selection of host mRNAs that are important for virus replication to persist and be translated [4,31].

As illustrated in Fig. 1B, poxviruses use virally encoded decapping proteins to remove the 5'-cap of the mRNA resulting in destabilization of the mRNA. A typical poxvirus, vaccinia virus (VACV) encodes two decapping proteins, D9 and D10, and these proteins likely maintain viral stage-specific protein synthesis as well as host cellular mRNA turnover [32,33]. Other viruses including bunyaviruses and orthomyxoviruses use cap-snatching mechanisms to remove the 5'-cap and use the removed cap to protect viral RNAs [8].

Intriguingly, prokaryotic viruses such as bacteriophages also trigger rapid host mRNA degradation. As shown in Fig. 1C, bacteriophage T4 Srd is demonstrated to stimulate the activity of *E. coli* essential endoribonuclease E via binding to its N-terminal in order to induce host mRNA degradation immediately after infection [34]. Though there is no homolog of Srd found yet in bacteriophage T2 and T7, host bulk mRNA degradation was also observed in T2- and T7- infected cells [7]. Little is known in the case of other bacteria such as *B. subtilis* yet, but host shutoff was observed in AR9 phage infected *B. subtilis* cells [35]. Further research is expected on their mRNA elimination after phage infection.

4. Conclusion and discussion

Influence of functional deficiency of viral factors that raises host mRNA turnover on viral replication has been studied for

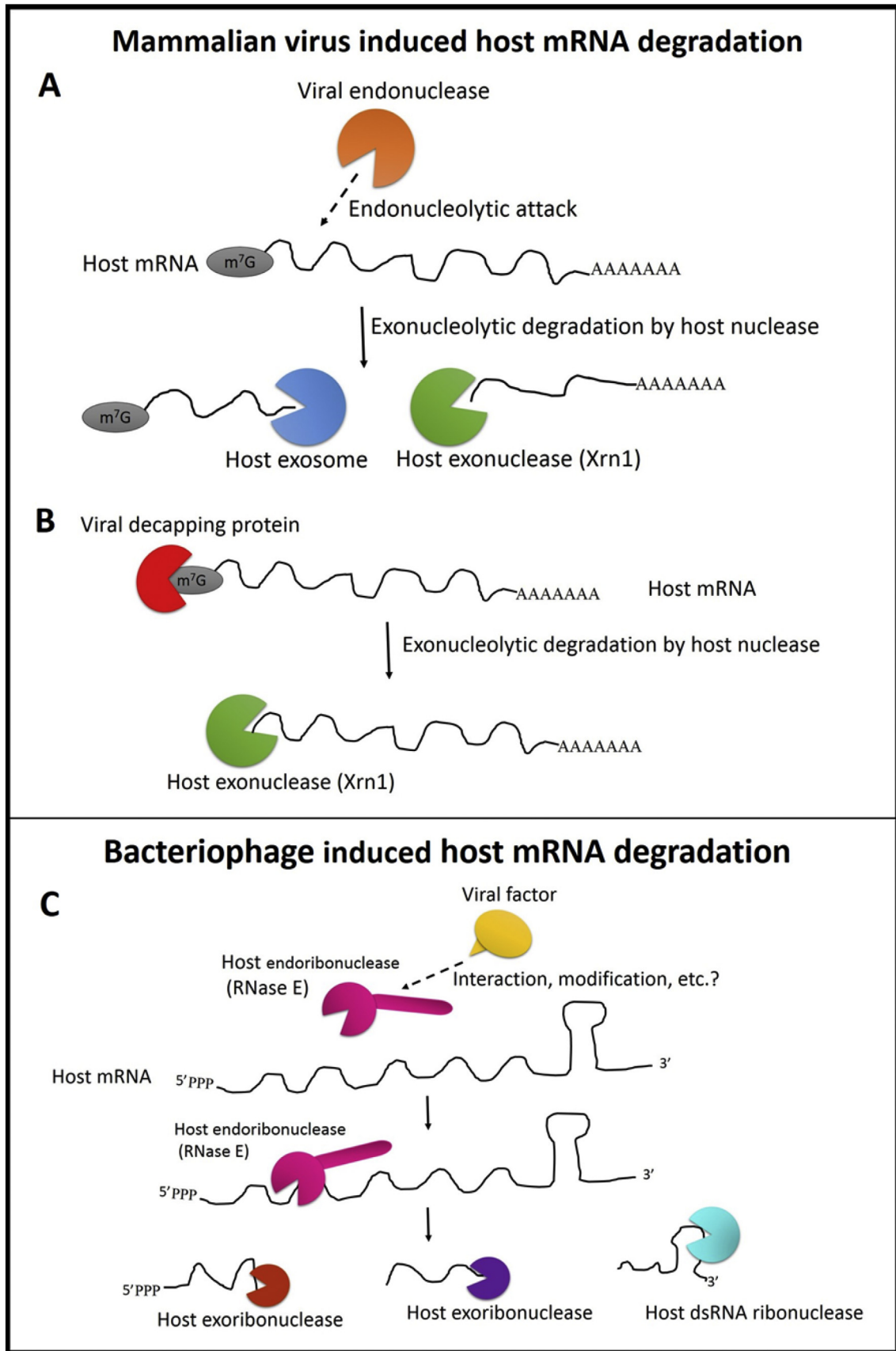


Fig. 1. Possible host mRNA degradation mechanisms induced by virus/bacteriophage infection [5,10,34].

years. Functional loss of viral factors mentioned above usually is not fatal for viral growth, but several pieces of evidence have been also obtained that global host mRNA turnover should be required for rapid transition of gene expression from host to viruses and efficient viral replication [16,34,36,37]. For instance, *vhs*-deficient mutant of HSV-1 results in low virulence and *Vhs* plays a role in evasion from non-specific host defense mechanisms during primary infection [16,36]. In addition, Matsuura's group showed that RNA replication of ScoV was low when *nsp1* was mutated in an *in vitro* RNA replica system [37]. Furthermore, from our own previous work, bacteriophage T4 *Srd* could stimulate the activity of host *E. coli* essential endonuclease E inducing bulk mRNA degradation and a deletion of *srd* causes inefficient propagation [34]. Therefore, it is likely to be a common mechanism in both prokaryote and eukaryote that infection with viral organisms activates the host mRNA degradation machinery for the shift of gene expression from host to virus and thus facilitates efficient viral propagation. How this contributes to offense immune response in mammalian cells is also an unanswered question so far.

5. Perspective

In this concise review, we mainly focus on virus-induced degradation of host RNAs during host shutoff, which helps to maximize viral gene expression and thus their replication.

Mammalian viruses as well as bacteriophages evolved unique mechanisms to propagate efficiently during infection. Host cells respond in order to create a hostile environment for viral replication, leading to the shutoff of mRNA translation (protein synthesis) and the assembly of RNA granules [38].

Recently, the HIV-1 unspliced mRNA has been shown to contain N⁶-methyladenosine (m⁶A), allowing the recruitment of YTH N⁶ methyladenosine RNA binding protein 2 (YTHDF2), which is involved in mRNA decay. This indicates that HIV-1 interacts with host mRNA decay components to accomplish viral replication successfully [39]. This finding implicates a novel mechanism involved in infection-induced host RNA degradation. In addition, viruses also employ numerous strategies to regulate the gene expression of both host and themselves despite of host shutoff. Hepatitis B Virus (HBV) mRNA interacting with host microRNA-122 (miR122) was reported to have an indispensable role for viral replication [40,41]. The interaction between viral mRNA and host microRNA is also known to be able to facilitate Hepatitis C Virus (HCV) replication [42].

Under development of imaging technologies, converting virus-host interaction signals to imaging visualization will help decipher the underlying cellular mechanisms. Single-molecule fluorescence imaging has been reported to monitor the entry, transport and virus-host cell interaction for some viruses like polio virus (PV) and Adeno-associated virus (AAV) [43]. Moreover, live cell imaging technology has also been reported for mRNA-protein interaction, which makes it possible to study cellular and viral gene expression directly. Yin et al. has constructed red color mCherry-based

trimolecular fluorescence complementation (TriFC) systems, visualized influenza A viral mRNA-protein interaction in living cells and helped understand more about IAV mRNA nuclear export mechanism [44]. They show that adapter proteins Aly and UAP56 are able to interact with three kinds of viral mRNAs while splicing factor 9G8 only interacts with one mRNA [44]. Therefore, it is expectable that application of advanced live cell imaging technology will facilitate in-depth research on virus-induced host mRNA degradation.

In summary, comprehensive understanding of virus-induced host shutoff and its connection in the whole process of viral replication as well as its interaction with host response through multiple technologies (*e.g.* molecular cell biological techniques and advanced imaging methods) is crucial to struggle with viruses. This could facilitate decoding virus-raised diseases and may provide new insights for both novel research tool and therapeutic strategy design for disease.

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References

- [1] Kamitani W, Huang C, Narayanan K, Lokugamage KG, Makino S. A two-pronged strategy to suppress host protein synthesis by SARS coronavirus Nsp1 protein. *Nat Struct Mol Biol* 2009;16(11):1134–40.
- [2] Kennell D. Inhibition of host protein synthesis during infection of *Escherichia coli* by bacteriophage T4. II. Induction of host messenger ribonucleic acid and its exclusion from polysomes. *J Virol* 1970;6(2): 208–17.
- [3] Sokoloski KJ, Wilusz CJ, Wilusz J. Viruses: overturning RNA turnover. *RNA Biol* 2006;3(4):140–4.
- [4] Mohr I. Closing in on the causes of host shutoff. *Elife*; 2016. p. 5.
- [5] Gaglia MM, Covarrubias S, Wong W, Glaunsinger BA. A common strategy for host RNA degradation by divergent viruses. *J Virol* 2012; 86(17):9527–30.
- [6] Svenson SB, Karlstrom OH. Bacteriophage T4-induced shut-off of host-specific translation. *J Virol* 1976;17(2):326–34.
- [7] Ueno H, Yonesaki T. Phage-induced change in the stability of mRNAs. *Virology* 2004;329(1):134–41.
- [8] Herbert KM, Nag A. A tale of two RNAs during viral infection: how viruses antagonize mRNAs and small non-coding RNAs in the host cell. *Viruses* 2016;8(6).
- [9] Abernathy E, Glaunsinger B. Emerging roles for RNA degradation in viral replication and antiviral defense. *Virology* 2015;479–480:600–8.
- [10] Narayanan K, Makino S. Interplay between viruses and host mRNA degradation. *Biochim Biophys Acta* 2013;1829(6–7):732–41.
- [11] Hui MP, Foley PL, Belasco JG. Messenger RNA degradation in bacterial cells. *Annu Rev Genet* 2014;48:537–59.
- [12] Mackie GA. RNase E: at the interface of bacterial RNA processing and decay. *Nat Rev Microbiol* 2013;11(1):45–57.
- [13] Deana A, Celesnik H, Belasco JG. The bacterial enzyme RppH triggers messenger RNA degradation by 5' pyrophosphate removal. *Nature* 2008; 451(7176):355–8.
- [14] Kwong AD, Kruper JA, Frenkel N. Herpes simplex virus virion host shutoff function. *J Virol* 1988;62(3):912–21.
- [15] Read GS, Frenkel N. Herpes simplex virus mutants defective in the virion-associated shutoff of host polypeptide synthesis and exhibiting

- abnormal synthesis of alpha (immediate early) viral polypeptides. *J Virol* 1983;46(2):498–512.
- [16] Suzutani T, Nagamine M, Shibaki T, Ogasawara M, Yoshida I, Daikoku T, et al. The role of the UL41 gene of herpes simplex virus type 1 in evasion of non-specific host defence mechanisms during primary infection. *J Gen Virol* 2000;81(Pt 7):1763–71.
- [17] Taddeo B, Zhang W, Roizman B. The herpes simplex virus host shutoff RNase degrades cellular and viral mRNAs made before infection but not viral mRNA made after infection. *J Virol* 2013;87(8):4516–22.
- [18] Page HG, Read GS. The virion host shutoff endonuclease (UL41) of herpes simplex virus interacts with the cellular cap-binding complex eIF4F. *J Virol* 2010;84(13):6886–90.
- [19] Glaunsinger B, Ganem D. Lytic KSHV infection inhibits host gene expression by accelerating global mRNA turnover. *Mol Cell* 2004;13(5):713–23.
- [20] Glaunsinger B, Chavez L, Ganem D. The exonuclease and host shutoff functions of the SOX protein of Kaposi's sarcoma-associated herpesvirus are genetically separable. *J Virol* 2005;79(12):7396–401.
- [21] Abernathy E, Gilbertson S, Alla R, Glaunsinger B. Viral nucleases induce an mRNA degradation-transcription feedback loop in mammalian cells. *Cell Host Microbe* 2015;18(2):243–53.
- [22] Mendez AS, Vogt C, Bohne J, Glaunsinger BA. Site specific target binding controls RNA cleavage efficiency by the Kaposi's sarcoma-associated herpesvirus endonuclease SOX. *bioRxiv preprint*; 2018.
- [23] Muller M, Glaunsinger BA. Nuclease escape elements protect messenger RNA against cleavage by multiple viral endonucleases. *PLoS Pathog* 2017;13(8). e1006593.
- [24] Feederle R, Bannert H, Lips H, Muller-Lantzsch N, Delecluse HJ. The Epstein-Barr virus alkaline exonuclease BGLF5 serves pleiotropic functions in virus replication. *J Virol* 2009;83(10):4952–62.
- [25] Rowe M, Glaunsinger B, van Leeuwen D, Zuo J, Sweetman D, Ganem D, et al. Host shutoff during productive Epstein-Barr virus infection is mediated by BGLF5 and may contribute to immune evasion. *Proc Natl Acad Sci USA* 2007;104(9):3366–71.
- [26] Covarrubias S, Richner JM, Clyde K, Lee YJ, Glaunsinger BA. Host shutoff is a conserved phenotype of gammaherpesvirus infection and is orchestrated exclusively from the cytoplasm. *J Virol* 2009;83(18):9554–66.
- [27] Huang C, Lokugamage KG, Rozovics JM, Narayanan K, Semler BL, Makino S. SARS coronavirus nsp1 protein induces template-dependent endonucleolytic cleavage of mRNAs: viral mRNAs are resistant to nsp1-induced RNA cleavage. *PLoS Pathog* 2011;7(12). e1002433.
- [28] Kamitani W, Narayanan K, Huang C, Lokugamage K, Ikegami T, Ito N, et al. Severe acute respiratory syndrome coronavirus nsp1 protein suppresses host gene expression by promoting host mRNA degradation. *Proc Natl Acad Sci USA* 2006;103(34):12885–90.
- [29] Khaperskyy DA, Schmaling S, Larkins-Ford J, McCormick C, Gaglia MM. Selective Degradation of Host RNA Polymerase II Transcripts by Influenza A Virus PA-X Host Shutoff Protein. *PLoS Pathog* 2016;12(2). e1005427.
- [30] Bercovich-Kinori A, Tai J, Gelbart IA, Shitrit A, Ben-Moshe S, Drori Y, et al. A systematic view on influenza induced host shutoff. *Elife*; 2016. p. 5.
- [31] Jan E, Mohr I, Walsh D. A cap-to-tail guide to mRNA translation strategies in virus-infected cells. *Annu Rev Virol* 2016;3(1):283–307.
- [32] Liu SW, Katsafanas GC, Liu R, Wyatt LS, Moss B. Poxvirus decapping enzymes enhance virulence by preventing the accumulation of dsRNA and the induction of innate antiviral responses. *Cell Host Microbe* 2015;17(3):320–31.
- [33] Shors T, Keck JG, Moss B. Down regulation of gene expression by the vaccinia virus D10 protein. *J Virol* 1999;73(1):791–6.
- [34] Qi D, Alawneh AM, Yonesaki T, Otsuka Y. Rapid degradation of host mRNAs by stimulation of RNase E activity by srD of bacteriophage T4. *Genetics* 2015;201(3):977–87.
- [35] Lavysh D, Sokolova M, Minakhin L, Yakunina M, Artamonova T, Kozyavkin S, et al. The genome of AR9, a giant transducing Bacillus phage encoding two multisubunit RNA polymerases. *Virology* 2016;495:185–96.
- [36] Strelow LI, Leib DA. Role of the virion host shutoff (vhs) of herpes simplex virus type 1 in latency and pathogenesis. *J Virol* 1995;69(11):6779–86.
- [37] Tanaka T, Kamitani W, DeDiego ML, Enjuanes L, Matsuura Y. Severe acute respiratory syndrome coronavirus nsp1 facilitates efficient propagation in cells through a specific translational shutoff of host mRNA. *J Virol* 2012;86(20):11128–37.
- [38] Poblete-Duran N, Prades-Perez Y, Vera-Otarola J, Soto-Rifo R, Valiente-Echeverria F. Who regulates whom? An overview of RNA granules and viral infections. *Viruses* 2016;8(7).
- [39] Toro-Ascuy D, Rojas-Araya B, Valiente-Echeverria F, Soto-Rifo R. Interactions between the HIV-1 unspliced mRNA and host mRNA decay machineries. *Viruses* 2016;8(11).
- [40] Hu J, Xu Y, Hao J, Wang S, Li C, Meng S. MiR-122 in hepatic function and liver diseases. *Protein Cell* 2012;3(5):364–71.
- [41] Li C, Wang Y, Wang S, Wu B, Hao J, Fan H, et al. Hepatitis B virus mRNA-mediated miR-122 inhibition upregulates PTTG1-binding protein, which promotes hepatocellular carcinoma tumor growth and cell invasion. *J Virol* 2013;87(4):2193–205.
- [42] Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science* 2005;309(5740):1577–81.
- [43] Banerjee S, Maurya S, Roy R. Single-molecule fluorescence imaging: generating insights into molecular interactions in virology. *J Biosci* 2018;43(3):519–40.
- [44] Yin J, Zhu D, Zhang Z, Wang W, Fan J, Men D, et al. Imaging of mRNA-protein interactions in live cells using novel mCherry trimolecular fluorescence complementation systems. *PLoS One* 2013;8(11). e80851.