RNA Viruses: RNA Roles in Pathogenesis, Coreplication and Viral Load

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Abstract: The review intends to present and recapitulate the current knowledge on the roles and importance of regulatory RNAs, such as microRNAs and small interfering RNAs, RNA binding proteins and enzymes processing RNAs or activated by RNAs, in cells infected by RNA viruses. The review focuses on how non-coding RNAs are involved in RNA virus replication, pathogenesis and host response, especially in retroviruses HIV, with examples of the mechanisms of action, transcriptional regulation, and promotion of increased stability of their targets or their degradation.

Keywords: Argonaute, DICER, HIV, RNA binding proteins, RNA secondary structure, RNA viruses, Small RNAs.

1. INTRODUCTION

RNA agents have been shown to play essential roles in evolution and regulation in all DNA/protein based life: based on RNA stem-loop secondary structures (built of paired stems and not-paired loops), pseudoknots, and loops with sequences showing affinity to target proteins. Group I and group II introns, viroids, viral (RNA and DNA viruses, bacteriophages, retrotransposons, Long Terminal Repeats) networks cooperate within cellular genomes as modular. Some non-coding RNAs have built complementary consortia, such as rRNAs, tRNAs, spliceosomes, editosomes, and other ribonucleoprotein particles (RNPs) [1]. Additionally, counterbalancing modules such as restriction/modification (RM) modules have evolved, assuring identity (self/non-self) of organisms. All fine-tuned steps of key cellular processes such as gene expression, transcription, translation, DNA recombination and repair, epigenetic imprinting, as well as various forms of innate and adaptive immunity, are essentially constituted by natural genetic content operators.

There are many reports showing that virus infection can alter the cellular microRNAs (miRNAs) to affect virus replication, some of them oppose the function of host restriction factors to enhance virus proliferation [2, 3]. Nowadays, researchers are trying to design miRNAs against virus proteins to control virus replication.

In the organisms, RNAs are associated to RNA binding proteins, helicases and RNases involved in RNA degradation and turnover.

This review discusses how these RNA interacting proteins and networks of regulatory RNAs are integrated into the general network of pathogenesis' control, especially during RNA virus infection.

1.1. RNA Viruses

Human diseases causing RNA viruses include Orthomyxoviruses, Hepatitis C Virus (HCV), Ebola disease, SARS, influenza, polio measles and retrovirus including adult Human T-cell lymphotropic virus type 1 (HTLV-1) and human immunodeficiency virus (HIV). RNA viruses have RNA as genetic material, that may be a single-stranded RNA or a double stranded RNA. Viruses may exploit the presence of RNA-dependent RNA polymerases for replication of their genomes or, in retroviruses, with two copies of single strand RNA genomes, reverse transcriptase produces viral DNA which can be integrated into the host DNA under its integrase function. Studies showed that endogenous retroviruses are long-terminal repeat (LTR)-type retroelements that account for approximately 10% of human or murine genomic DNA.

Among human retroviruses, HIV-1 is a lentivirus with an RNA genome formed by two copies of a single-stranded, positive-sense RNA. The HIV-1 RNA genome is associated to the nucleocapsid protein (NC) and to viral enzymes, thus it is "protected" within the viral capsid mainly formed by the p24 protein. Upon entry into the target cell, the viral RNA genome is reverse transcribed into double-stranded DNA by a virally encoded reverse transcriptase that is transported along with the viral genome into the virus particle. The viral DNA is imported into the cell nucleus and integrated into the cellular DNA by a virally encoded integrase and host cofactors. Once integrated, the virus may become latent, or may be transcribed, producing new RNA genomes and viral proteins that are packaged and released from the infected cell as new virus particles that will infect other cells to begin the new replication cycle. Many aspects of the life cycle of retroviruses are intimately linked to the functions of cellular

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proteins and RNAs. HIV-1 and Moloney Murine Leukemia Virus (MoMuLV) have been studied for the dimerization of two RNAs.

1.2. Retrotransposons, LTRs, Retrotranscriptases

Long Interspersed Nuclear Elements-1 (LINE-1) and endogenous retroviruses (HERVs) encode reverse transcriptase (RT) proteins in vertebrates. LINE-1s (L1s), the most studied, active autonomous mobile DNA in humans, accounts for about 17% of human DNA while HERVs account for about 8% and, together with the non-autonomous SINE/Alu family (about 10%) constitute a large proportion of the human genome. L1s encode two open reading frames (ORFs 1 and 2). The shorter ORF1 translation product (ORF1p) is an RNA binding protein, thought to also bind to non-retroviral transcripts, protects against nuclease degradation and specify nuclear import of the ribonuclear protein complex (RNP). ORF2 encodes a multifunctional protein (ORF2p) comprising apurinic/apyrimidinic endonuclease (APE) and reverse-transcriptase (RT) activities, responsible for retroelement's replication and their integration into chromosomal DNA. However, some clades of APE-type retroelements only encode a single ORF-corresponding to the multifunctional ORF2p [4]. HERVs, closely resembling infectious retroviruses, have mutated and/or truncated provirus structures and have lost their ability to replicate or retrotranspose. Nonetheless, proteins encoded by different types of HERVs are still exerting biological activities and most of the HERV-associated regulatory regions, termed "long terminal repeats" (LTRs), preserve their functions as a promoter–enhancer region. Functional "awakening" of HERVs and LTRs from their epigenetic silencing can play causative roles in tumorigenesis; in particular, HERV-K (HML-2), the most recently integrated family with a nearly complete retroviral structure, is involved in neoplastic and autoimmune pathological processes [5]. Retroelements, which mobilize throughout the genomes by a copy-and-paste process involving RNA intermediates, have the potential to modify mammalian genomes not only through insertional mutagenesis yet generating many other novelties that alter genomes both structurally and functionally [6]. Not surprisingly, cells have adopted strategies aiming at restricting the mobility and deleterious consequences of uncontrolled retrotransposition [6].

Although heavily mutagenic and responsible for deleterious gene disruptions, retroelements may have provided some beneficial genomic functions with potential evolutionary advantages.

Incubation of mouse zygotes with 5′-bromodeoxyuridine (BrdU) yields massive incorporation of this nucleoside analogue in newly synthesized DNA; surprisingly, a significant incorporation still occurs in both zygotic pronuclei in the presence of aphidicolin, a specific inhibitor of DNA replication. This aphidicolin-resistant BrdU incorporation is quantitatively abolished when embryos are simultaneously exposed to abacavir, a nucleoside RT inhibitor, thus revealing its RT-dependent nature. Moreover, quantitative PCR analysis showed that LINE-1 copies are newly synthesized at the zygote- and two-cell embryo stages and nearly doubled compared to gamete copy number. These findings support the conclusion that RT-dependent amplification of LINE-1 retro-

transposons is a distinctive feature of early embryonic genomes [7].

Inhibition of RT activity in cancer cell lines, either by LINE-1-specific RNA interference or by RT inhibitory drugs, was found to reduce proliferation and promote differentiation and to antagonize tumor growth in animal models. Using CsCl density gradients, Alu- and LINE-1-containing DNA:RNA hybrid structures were identified in cancer yet not in normal cell lines [8]. In cancer cells the highly abundant RT activity intercepts various RNA classes and reverse transcribes them generating RNA:DNA hybrids. This may impair the formation of double-stranded RNAs required in the production of small regulatory RNAs (miRNA in particular), with a direct impact on gene expression. RT inhibition restores the 'normal' small RNA profile and the regulatory networks that depend on them. Thus, the retrotransposon-encoded RT drives a previously unrecognized mechanism crucial to the transformed state in tumor cells.

Recent computational studies confirm the association between L1 expression and the generation of small RNAs. L1 expression seems to have a role in the activation of small RNA expression as emerged comparing data from L1-active and L1-silenced breast cancer cells. Cells in which L1 expression was silenced greatly increased the expression of a number of miRNAs, in particular members of the let-7 family, few piwiRNAs and several repeat-RNAs targeting LTRs, LINEs and SINE elements [9].

1.3. Small RNAs, miRNAs, RNA Interference and Immunity

miRNAs have become a prototype of several classes of small RNAs (sRNAs) [10]. These sRNAs act as single strand filaments incorporated into the RNA induced silencing complexes (RISC) acting as guides to Argonaute (Ago) enzymes degrading the target RNA complementary to these sRNAs. The processing into mature sRNAs requires protein complexes containing endoribonucleases: DROSHA in the nucleus, cleaving the dsRNA precursors into pre-miRNAs. The pre-miRNA hairpin structure is then exported into the cytoplasm via exportin-5. In the cytoplasm it assembles in RNA induced silencing complex (RISC) which includes Dicer ribonucleases that cleave pre-miRNAs into mature single strand sRNAs, Ago enzymes cleaving the mRNAs with sequences complementary to the microRNAs [11], and other associated proteins, such as PW182/Argonaute-2, a 182 kDa RNAse associated to P-bodies [12], HIV transactivating response RNA-binding protein (TRBP) [13], and fragile X mental retardation protein (FMRP1) [14]. Mature miRNAs play important roles in the regulation of mammalian genes. It has been suggested that over 30% of all human genes are regulated by miRNAs. While recent genome-wide siRNA and shRNA screenings have shown that several hundred host cell proteins contribute to the regulation of HIV-1 infection in human cells, how miRNA-mediated regulation complements this picture is poorly understood. Other processing complexes are involved in the production of sRNA with various sizes: piwiRNA and siRNA, with size ranging from 21-nt to 25-nt, is the output of this processes. Their role is in the amplification of signals such as RNA primed RNA amplification, and the spreading of anti-viral RNA interference mechanism.

1.4. RNA Pseudoknots, Hairpins and Secondary Structures

RNA can both store information in its linear sequence and take on critical structural and catalytic roles in the cell, such as during the translation of messenger RNA into proteins [15]. These latter functions depend on the complex higher-order structures RNA is able to form. Homan *et al.* reported a method to probe the intricate conformational states in the analysis of HIV-1 NL4-3 RNA genome [15]. They chemically modified exposed segments of three complex RNA structures. They then sequenced the RNA to map the locations of the multiple modifications in each individual linear RNA molecule. This allowed the researchers to deduce interactions in three-dimensional space, and to uncover the local conformation, providing valuable information on the folding and function of RNAs [15]. Single-molecule RNA structure was tagged, i.e. multiple sites were chemically modified are identified by massively parallel sequencing of single RNA strands, and then analyzed for correlated and clustered interactions. The strategy thus identified RNA interaction groups by mutational profiling (RING-MaP) and made possible two applications. Firstly, through space interactions, 3D models were created for RNAs, spanning 80–265 nucleotides, and intramolecular interactions that stabilize RNA were characterized. Secondly, distinct conformations in solution were identified and revealed previously undetected hidden states and large-scale structural reconfigurations that occur in unfolded RNAs relative to native states. RING-MaP analysis of single-molecule nucleic acid structure enabled a novel view of the global architecture and multiple conformations that govern the functions in RNAs.

Additional methodologies that have been used in testing the secondary structure of RNA genomes have been published. 2'-hydroxy acylation of RNA was analysed by primer extension and mutational profiling (SHAPE-MaP) [16] and used to define a new model of HIV-1 RNA genome.

Advances in RNA structure prediction from sequence are currently made by setting and testing new tools for generating hypotheses and confirming viral RNA structure-function relationships [17]. On this basis, novel methods have been tested to investigate the sequence-dependence of RNA-protein interactions [18]. RNA substrates demonstrate diverse intramolecular interactions, including mismatched base bulges, stem loops, pseudoknots, g-quartets, divalent cation interactions and noncanonical base pairs, determining three-dimensional RNA structure. The molecular evolution of MS2 from low- to high-affinity hairpins, was analysed and quantified. The results suggest that quantitative analysis of RNA on a massively parallel array (RNA-MaP) provided an insight into the biophysics of RNAs and on consequences of sequence-function relationships.

Several RNA secondary structures have been shown important for the virus functions: internal ribosomal entry structure, internal ribosomal entry site, and 5' UTRs regulate the start of translation of operons. For example, in influenza virus type C, there are seven vRNA segments with non-coding regions (NCR) at the extremities, that affects transcription and replication, by the type-C and type-A polymerase complexes [19]. To determine the molecular structure adopted by these NCR, various bioinformatics tools, including RNAfold, RNAstructure, Sfold, and Mfold, have been used. Various nucleotide polymorphisms (SNPs) in these non-coding regions may differentiate infective strains, such as major or minor read-through activity and differential expression of ORFs in operons. In Orthomyxoviridae, such as human influenza viruses or infective salmon anemia virus (ISAV), studies suggest an association between the molecular architecture of NCR regions and their role in the viral life cycle [20]. The 3' and 5'-terminal sequences of influenza A, B and C virus RNA segments are highly conserved and show partial inverted complementarity [21]. The viral RNA 3'- and 5'-end structure and mRNA transcription of infectious salmon anaemia virus resemble those of influenza viruses [22]. The aligned Non-Coding Region (NCR) sequences from ISAV isolates were compared with those from influenza virus, and consensus sequences were found, based on conserved regions identified in the consensus sequence [23]. This hypothetical structure, together with a comparison with influenza viruses, yielded reliable secondary structure models that lead to identification of conserved nucleotide positions at inter-genus level to determine which nucleotide positions are involved in the recognition of the vRNA/cRNA by RNA-dependent RNA polymerase (RdRp) or mRNA by the ribosome. The NCR contain conserved sequences that vary in length among the various genera of the family Orthomyxoviridae [24]. It has been reported that the first 12 and 13 nucleotides correspond to conserved sequences in the 3' and 5' ends, respectively, of all segments of the influenza A vRNA [25]. Structurally, these conserved sequences in influenza A have been described as partially complementary and capable of interacting in cis within each segment of RNA, forming structures called panhandles [26, 27] In Orthomyxoviruses, transcription of the genome requires the vRNA to act as template for each genomic segment, and for transcription to occur, the conformation adopted via the folding of the NCR is essential [26].

1.5. HIV-1: Host Factors Supporting From the Entry to Virus Replication

Recently importin 7 and importin α have been shown to enhance nuclear entry of HIV-1 (but not HIV-2) correlating with its ability to bind to the viral integrase and the virus accessory protein Vpr which are components of pre-integration complex (PIC) [28, 29]. Transportin 2, identified using siRNA screens [30-32], is also able to enhance nuclear import of PIC. The most important finding was that tRNA molecules themselves can act as nuclear entry chaperones for the HIV PIC. HIV-1 transcription is regulated by the viral promoter located in the 5′LTR of the provirus. The LTR contains binding sites for several transcription factors such as Sp1 and NF-κB, NFAT, LEF-1, COUP-TF, Ets1, USF and AP-1 [33].

The RNA binding protein Staufen appears to act as a chaperone to the RNA and has been detected in viral particles. Similarities between this and the known HIV TAR RNA binding protein TRBP may promote further investigations.

RNA cap methylases are cellular factors that regulate post transcriptional HIV-1 RNA expression in order to pro-

duce a viral mRNA camouflaged by cellular mRNA showing a 7-methylguanosine (m7G) cap.

1.6. RNA Silencing and Host-virus Interaction

Plants and lower eukaryotes produce miRNAs and siR-NAs as a form of RNA-interference (RNAi) to restrict infecting viruses. While mammals conserve the same functional miRNA repertoire and RNA-silencing machinery, some have debated whether they employ a miRNA-based antiviral strategy. For endogenous mammalian retroviruses, there is a large body of literature demonstrating that a variety of small non-coding RNA forms are employed to silence these elements. *In silico* analyses have also indicated that exogenous mammalian viruses may be similarly susceptible to miRNA-based restriction. The notion that miRNAs restrict viruses in mammals as they do in invertebrate or plant cells is supported by increasing examples of RNAi-silencing suppressors encoded by mammalian viruses such as Adenovirus, HCV, Ebola, Influenza A virus, primate foamy virus, HIV, SARS corona virus and HTLV-1. Further investigation is needed to understand how RNA-based and protein-based viral restriction mechanisms cooperate together in human cells.

Considering tumour- associated viruses (oncoviruses), it is estimated that 20% of all cancers are linked to infectious agents. Studies of oncogenic DNA viruses have contributed to the understanding of key molecular mechanisms of tumorigenesis and viral oncogenicity [10]. Virally encoded oncoproteins such as adenovirus E1A and human papillomavirus (HPV) E7 can bind an array of cellular proteins to override proliferation arrest. Adenovirus VA1 noncoding RNA can inhibit small interfering RNA and microRNA biogenesis, both by inhibiting nuclear export of shRNA or pre-miRNA precursors, competing for the Exportin 5 nuclear export factor, and inhibiting Dicer function by direct binding to Dicer [10]. Recently, many viral-encoded miRNAs have been discovered, especially abundant in viruses transcribed from double-stranded DNA genomes. Several virus-encoded miRNAs have unique aspects to their biogenesis, such as the location within the precursor transcript.

Bovine leukemia virus, a member of the retrovirus family, was the first RNA virus shown to synthesize a viral RNA that is proficiently processed in cells into small ncRNAs [34], producing numerous miRNAs. BLV avoids Drosha-mediated cleavage of its genome and mRNAs, which overlap the miRNA cluster, since BLV miRNAs, unlike most known miRNAs, are encoded as shorter RNA polymerase III (pol III) transcribed hairpins that can directly serve as Dicer substrates. Thus, BLV transcripts are not cleaved by Drosha, while subgenomic small RNAs are processed into miRNAs.

Influenza A virus replicates its genome in the nucleus and is exposed to the nuclear microRNA processing factors Drosha and DGCR8. At 8 hours after infection, 18- to 27-nt small viral leader RNAs (leRNAs) bearing a 5'-terminal triphosphate are produced from the 5' ends of all eight influenza virus genomic RNA (vRNA) segments [35]. The high-level production of leRNAs may imply a role in the regulation of the switch from viral mRNA transcription to genomic RNA synthesis.

It is generally believed that cytoplasmic RNA viruses do not encode miRNAs, owing to inaccessible cellular miRNA processing machinery. In a genome-wide analysis and identification of miRNAs originating from hepatitis A virus (HAV), a typical cytoplasmic RNA virus [36], two novel virally encoded miRNAs, hav-miR-1-5p and hav-miR-2-5p, were identified, generated from viral miRNA precursors (pre-miRNA).

Presently, functions have been proposed for viral miRNAs from three different viral families: herpesviruses, polyomaviruses and retroviruses.

Four vsRNAs were detected in enterovirus 71-infected cells using next-generation sequencing and northern blots. Viral infection produced substantial levels ($>10^5$ copy numbers per cell) of vsRNA1, one of the four vsRNAs. Dicer was shown to be involved in vsRNA1 generation in infected cells. vsRNA1 overexpression inhibited viral translation and IRES activity in infected cells. Conversely, blocking vsRNA1 enhanced viral yield and viral protein synthesis. vsRNA1 targets stem-loop II of the viral 5' untranslated region and inhibits the activity of the IRES through this sequence-specific targeting [37].

Websites and databases are available that classify most of the small RNAs and mRNAs produced by virus families [38]; In VIRMir database four miRNAs are recorded derived from HIV genome hiv1-miR-tar-5p, hiv1-miR-tar-3p, hiv1-miR-n367, originating from *nef* gene and targeting nef mRNA [39], hiv1-miR-h1 [40]. In addition to these four, another one, hiv1-miRH3, was reported more recently [41], which locates in the mRNA region encoding the active centre of reverse transcriptase (RT), targets HIV 5'-LTR and binds to the TATA box, upregulating promoter activity [41].

The HIV-1 Trans-Activation Response (TAR) element is a hairpin structure of ~50 nucleotides found at the 5' end of the HIV viral mRNA. TAR element is recognized by the RNAi machinery and it has been shown to be processed by Dicer yielding a viral miRNA involved in chromatin remodelling of the viral LTR [42] and targeting the apoptosis genes ERCC1 and IER3 [43, 44]. This viral miRNA is detectable in infected cells and appears to contribute to viral latency.

In a recent publication [45] numerous small RNAs were found deriving from HIV-1 RNA genome. Most of the sequences, with positive polarity (98.1%) could be structured RNAs (sRNAs) or miRNA-like (vmiRNAs). A small portion of the viRNAs, with negative polarity, is encoded within the 3′-UTR. These viral siRNAs (vsiRNAs) were shown to act inhibiting virus replication, since their inhibition using antagomiRs increases virus replication. Three of the HIV-1 small RNAs were shown to be processed by the RNAi machinery. There are data showing that HIV-1 can express an antisense transcript from the 3'-end of its genome that forms long RNA duplexes with counterpart sense HIV-1 RNAs [46-48]. Most HIV sRNAs are not supposed to function as miRNAs, because of lack of evolutionary conservation amongst strains, but may still assume a hairpin structure in the regions containing the conserved bases.

1.6.1. Host miRNAs Deregulated by Virus Infection

Presently, viral interactions with cellular miRNAs have been identified, expanding the knowledge of miRNA functions [49].

One of the first host miRNAs shown to block retrovirus was miR-32, effectively limiting primate foamy virus type 1 (PFV-1) replication [50].

Inhibition of influenza virus replication has been described for four miRNAs: miR-323, miR-491, miR-654, and let-7c. Vesicular stomatitis virus is inhibited by miR-24 and miR-93, hepatitis B virus by miR-125a-5p, miR-199a-3p and miR-210; and HCV by miR-196, miR-296, miR-351, miR-431, and miR-448 [51]. In the case of HCV, a liver-specific miRNA, miR-122, was found to directly target HCV RNA sequence to up-regulate viral replication [52].

Substantial advances have been made in the understanding of the interplay between HIV-1 and the cell's RNAi activity. HIV-1 infection can change the miRNA expression profiles in the circulating blood cells from infected individuals [51].

Host miRNAs can modulate HIV replication either directly by targeting HIV RNA, or targeting the mRNAs that encode host cell factors relevant to HIV replication. miR-217 was found induced by Tat and increased HIV-1 expression by targeting sirtuin-1 (SIRT-1) with deacetylase activity inactivating Tat function [53]. miR-198 was shown to inhibit HIV-1 gene expression and replication in monocytes, action linked to its down-regulation of cyclin T1 [54].

Recent reports studied several human miRNAs targeting HIV-1 sequences. Using target prediction software, five miRNA (miR-29a, miR-29b, miR-149, miR-324-5p, and miR-378) were found to target sequences, two of them located in the viral nef gene, of the HIV-1 genome [55]. miR-29a was shown to inhibit nef expression, and to repress HIV replication in Jurkat cells. Recently inhibition of HIV-1 infection by miR-29a and miR-29b was confirmed [56, 57], however, HIV-1 is protected by a complex RNA secondary structure surrounding the target site. A different group of five miRNAs (miR-28, miR-125b, miR-150, miR-223, and miR-382) that target the 3'-UTR of the HIV genome was reported [58]. These "anti-HIV" miRNAs were shown to be enriched in resting CD4+ T cells and were hypothesized to be involved in proviral latency. In another study, four of these miRNAs were found responsible for differences between monocytes and macrophages in their permissivity to HIV infection [59]. Recently the action of miR-29, miR-133b, miR-138, miR-149 and miR-326, targeting HIV-1 sequences, was shown [60]. Therefore, in divergent cells and in varying contexts different miRNAs may selectively regulate HIV-1 infection through direct targeting viral sequences. Thus, a complex set of miRNA-mediated positive and negative regulatory events is influencing viral replication [51]. In monocytes, miR-1236 was shown to inhibit HIV-1 infection by repressing translation of cellular factor Vpr binding protein, VprBP/DCAF1 [61].

A significant number of host non-coding RNAs have been found in Hepatocellular carcinoma (HCC) caused by HCV infection, and are involved in pathogenesis of HCV and HCV-induced HCC [62].

1.7. Subversion of IFN Responses

The role of type I-IFNs in increasing host susceptibility could be explained by modulation of components of the immune response involved in controlling the growth of infective agents, such as induction of T cell apoptosis, resulting in greater IL-10 secretion by phagocytic cells, in turn dampening the innate immune response. A mechanism by which viruses survive inside cells is by inactivating the cellular antiviral machinery, or inactivating the RNA interference response, acting on the dsRNA-activated protein kinase (PKR). Infection thus can escape from the immune response by deregulation of the interferon signaling and the processes forming small RNAs acting in RNA silencing pathways.

1.8. Virus Deregulation of Stress Granule Function

It was shown that viral life cycle within cells involves hijacking cellular processes and nuclear targeting. This is also at the base of redistribution of translation machinery during the stress response involving the formation of stress granules, processing bodies (P-bodies, PB), and perinuclear paraspeckles. During oxidative stress, arsenite, or by phosphorylation of eIF2α, cells undergo a translation arrest, stalling the RNAs in the form of ternary complexes that include eukaryotic initiation factor 2 (eIF2)/GTP/Met-tRNA. These stress granules, containing the RNA to be translated, have a role in spatial and temporal inhibition of mRNAs, until resolving the stress for processing the mRNAs, or degrading it in case of non recovery from the stress. Stress granules are formed by a nucleation process that involves several principal factors, such as the RNA decay factor G3BP, which prevents the localisation of ribosome and initiation factors in silenced SG foci, the translational suppressor TIA1, TIAR, Caprin1, USP10, DDX6 (Rck/p54), DDX3 helicase, poly-A binding protein PAPB and Lsm1. Additionally, SGs contain enzymes of the RNA silencing pathway, such as Argoanute-2, trans-acting factors, Hsp90 complexes and RNA binding proteins, found at the site of small RNA-mediated repression of RNA targets [63].

Different viruses exploit the binding to protein scaffolds to avoid SG formation or to assemble their RNA into SGs devoid of cellular RNA, thus exploiting the transcriptional machinery for their own means [64]. In RNA viruses, the knowledge has increased recently, especially focusing on HCV infected cells, where SG and P-body components are relocalised to the periphery of lipid droplets, and an oscillation between SG assembly and disassembly is observed upon interferon I treatment, depending on the inhibition of PKR by the eIF2 phosphatase GADD34 [65]. West Nile Virus (WNV) inhibits SG formation by scavenging Reactive Oxygen Species (ROS), and also relocalising the SG scaffolding proteins into perinuclear foci where WNV replication occurs by exploiting cell translation machinery. HIV-1 blocks SG assembly *in vitro* and *ex vivo* in patient samples. Gag has an important role in inhibition of SG, dependent on the interaction between host factors EIF2 and G3BP1. Influenza A virus (IAV) proteins can block SG formation: IAV polymerase complexes function in the nuclei of infected cells, generating mRNAs with a 5' cap and polyA-tail that are transferred into the cytoplasm for translation. Non-structural protein 1 (NS1) inactivates PKR, preventing eIF2 phoshorylation; nucleoprotein (NP) inhibits SG formation through eIF independent mechanisms; host-shutoff protein polymerase-acidic protein-X (PA-X) is essential to block SG formation. Measles virus infection progresses through the synthesis of 5'-copyback

defective-interfering RNA (DI-RNAs), that are complementary in the 5' and 3' termini, forming double stranded RNAs, efficient in activation of PKR and PKR signaling. Downstream to this event, measles protein C is required for alleviation of SG translation inhibition, while A-to-C mutation events dependent on ADAR modify the virus genome. In picornaviruses, such as enteroviruses, proteinases have been shown involved in disassembly of SG, while in kobuviruses other factors, such as a small leader peptide, are important in SG inhibition.

1.8.1. Vpr/Vpx

The viral accessory protein Vpr is a component of the PIC. It is reported that Vpr is important on the nuclear import of PIC by interacting with a nuclear pore protein, importin α [29]. Since PIC is larger than a nuclear pore, various other nuclear pore complex proteins have also been identified in PIC nuclear entry, including Nup 98, Nup 124p, Nup 358 and Nup 153.

Vpr belongs to the RAD23-like family of proteins, similarly to Vpx [66]. Vpr is a chaperone that guides target proteins to bind to VprVP/DCAF1, a receptor of the CULLIN E3 ubiquitin ligase (Cul4-DDB1, Cul5) [67-69]. Vpx is a small virion-associated adaptor protein encoded by viruses of the HIV-2/SIVsm lineage of primate lentiviruses, a Vpr paralogue, that enables these viruses to infect monocyte-derived cells.

One of the main activities of Vpr/Vpx is the degradation of target proteins through binding to VprBP, thus recruiting the 26S proteasomal pathway. Several studies showed the potentiality of Vpr to interact with many E2 and E3 enzymes [70]. Vpr has been shown to affect, either directly or indirectly, the modification of proteins, such as ubiquitinylation, phosphorylation and neddylation. In this way, Vpr influences and regulates the levels of many proteins [71].

Vpr has been found to affect the levels of few miRNAs among which is miR-34a, as well as the genes IRBIT, SERP1, SIRT1, NEFM, Drp-1, Orai, STIM1, IP3R and CREB [72]. It was reported that Vpr inhibits short hairpin RNA function as expected upon reduction of endoribonuclease Dicer levels by binding with VprBP to block maturation of miRNAs [73].

Also it was reported that Vpr can interact with spliceosomal protein SAP145 to mediate cellular pre-mRNA splicing inhibition. Although the mechanism is not clarified, Vpr is sufficient alone to promote HCV RNA replication [74]. Vpr is reported to be a component of the reverse transcription complex (RTC) and co-localizes with the viral nucleic acid and integrase within purified HIV-1 RTCs.

Vpr also can regulate several proteins and host factors, some of them can affect RNA replication. The most interesting are TERT [67], the type I interferon regulatory factor 3 (IRF-3) [75], A3G [76], TRIM proteins [77], uracil DNA glycosylase 2 (UNG2) [78] and single strand selective monofunctional uracil-DNA glycosylase 1 (SMUG1). A premature activation of the SLX4 complex has been shown dependent on Vpr, promoting G2/M arrest and escape from innate immune sensing [79].

Zahoor *et al.* using microarray system found that Vpr protein enhanced the mRNA level of interferon (IFN)-stimulated genes (ISGs), and causes phosphorylation of STAT1 at tyrosine 701 in human monocyte-derived macrophages (MDMs) infected with a recombinant adenovirus expressing Vpr [80]. These findings enhance the current understanding of HIV-1 replication and pathogenesis in human macrophages. Vpr, together with other HIV factors, recruits cellular adaptors to facilitate immune evasion [81]. HIV-1 Vpr differentially regulates the expression levels of chemotactic cytokines such as CXCL1, CXCL5, CXCL7, CXCL9, CXCL10, and CXCL11. A report showed that CXCL10 and CXCL11 are up-regulated in HIV-1-infected macrophages and play a key role in the recruitment and spread of HIV-1 to susceptible CD4+ T-cells [82].

1.9. Intracellular Defences Against HIV

Clearly the cell is not a passive participant in virus replication. In addition to the cellular pathways subverted by the virus for its own use there are inhibitory factors within cells which act as intracellular defences and whose presence inhibits or 'restricts' the virus. The first one, identified in retrovirus infected cells, was Fv1 which restricts ecotropic murine leukemia viruses. Following this finding, other similar factors restricting HIV were identified. Because of their potential importance in novel antiviral approaches, they have been extensively investigated in recent years. APOBEC3 family [83], TRIM family [77, 84], Tetherin [85], IRF3 [75], SMUG1, UNG2 [86], SAMHD1 [87] and SLX4 [79] were well known for being involved in HIV infectivity, indicating that the viral proteins can interact and modulate their activity.

As an adaptive response, viruses develop the ability to interact and deactivate these defences, a mechanism named pathogen mimicry. Among several mechanisms, there are: a) the development of proteins and molecules that act interfering with cellular processes; b) virus miRNA analogs of host miRNAs, exploiting the presence of a network of cell effectors and antiapoptotic factors; c) incorporating protein-protein interaction domains or association modules in their genome; d) through increased mutation rates evolving the recognition domains of proteins targeted by cellular defences.

1.9.1. Epigenetics. Resetting of Epigenetic Marks

Infective agents and bacteria when entering inside the cells activate several mechanisms to avoid immune detection [84]. Many viruses entering inside the cells are able to derail the cellular machinery, including the epigenetic control. A large set of host proteins required for HIV infection have been identified through a functional genomic screen [88]. RNAi screens have been performed for host factors required for HIV replication [30].

HIV-1 integration is generally random but it has more easier access into active genes; however, independent of the site of integration in human chromosomes, two nucleosomes, named nuc-0 and nuc-1, are precisely organized in the 5′LTR. In particular, the histone organized nuc-1 structure (located at position −2 to +140 of the LTR) normally serves to down modulate basal transcription.

The HIV-1 transcriptional activator Tat has evolved mechanisms to resolve the transcription block. Tat is associated with histone acetyl transferase (HAT) proteins whose activities remodel nucleosomes to allow transcriptional access. Tat has been shown to bind several different HATs: CBP/p300, p/CAF, GCN5, Tip60, and TAFII250. Through binding to the HAT proteins, Tat relieves chromatin repression at the HIV-1 LTR. Recently, Tat has also been found to bind a histone chaperone protein, hNAP-1, which acts with ATP-dependent chromatin remodeling complexes to facilitate transcription.

Counteracting the effect of HATs, the histone deacetylase proteins (HDAC) remove the acetyl-group from HAT-acetylated histones to enforce transcriptional silencing. In the HIV-1 LTR, it is thought that the LSF protein binds at position −10 to +27 of the LTR to recruit the YY1 factor which further binds HDAC-1 to silence viral transcription. Tat expression down regulates HDAC-1 to remove the transcription repression. A similar recovery from repressive inhibition has been obtained through treatment with HDAC inhibitors (HDACIs) such as Trichostatin A (TSA), Valproic Acid (VPA), and sodium butyrate.

1.10. Conclusion

In this review we highlighted the importance of cellular and viral RNAs in the cell response to RNA viruses, especially to retroviruses and endogenous L1 remnants of viral DNA integration. In addition, we reviewed several pathways involving small RNAs and short interfering RNAs deregulated in various states, from active infection to virus-associated cancers and defective immune signaling. A special role has been assigned to the deregulation of interferon response and the inhibition of protein complexes in stress granules and P-bodies, RNA binding proteins, RISC components and the RNA silencing machinery.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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