

REVIEW



# Involvement of the nucleolus in replication of human viruses

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## SUMMARY

Viruses are intracellular pathogens that have to usurp some of the cellular machineries to provide an optimal environment for their own replication. An increasing number of reports reveal that many viruses induce modifications of nuclear substructures including nucleoli, whether they replicate or not in the nucleus of infected cells. Indeed, during infection of cells with various types of human viruses, nucleoli undergo important morphological modifications. A large number of viral components traffic to and from the nucleolus where they interact with different cellular and/or viral factors, numerous host nucleolar proteins are redistributed in other cell compartments or are modified and some cellular proteins are delocalised in the nucleolus of infected cells. Well-documented studies have established that several of these nucleolar modifications play a role in some steps of the viral cycle, and also in fundamental cellular pathways. The nucleolus itself is the place where several essential steps of the viral cycle take place. In other cases, viruses divert host nucleolar proteins from their known functions in order to exert new unexpected role(s).  
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## INTRODUCTION

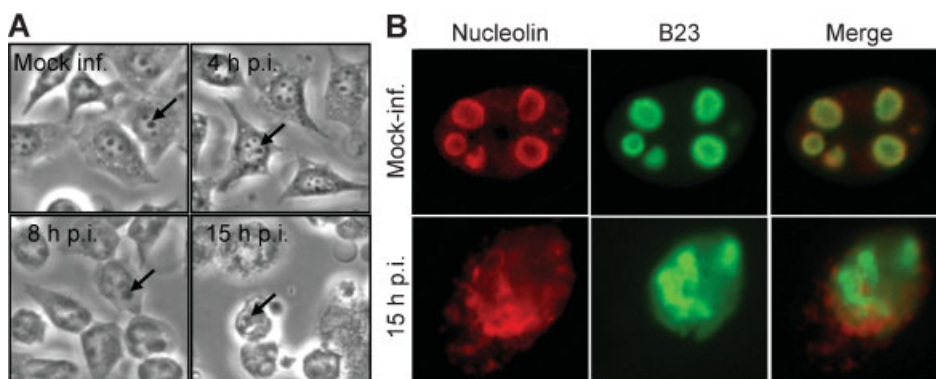
The analysis of the interactions between a virus and its host cell is critical for understanding the viral cycle, the physiopathology of viral infections and for improving the development of novel and rationally designed antiviral strategies. Viruses

are intracellular pathogens with small genomes and, therefore, they have to divert some of the cellular machineries to provide an optimal environment for their own replication. Many viruses, whether they replicate or not in the nucleus of infected cells, induce modifications of nuclear substructures, including chromosomal domains, promyelocytic leukaemia (PML) bodies, Cajal bodies and nucleoli [1]. The nucleolus, which is the best-studied nuclear body, forms around the clusters of genes coding for ribosomal RNA (rRNA), and is the site of rDNA transcription, rRNA processing and ribosome assembly. Indeed, until recently, nucleoli were considered essentially as the sites of ribosome biogenesis. However, several insights from the last decade are changing our understanding of the biology of nucleoli. Advances in proteomics and in live cell imaging have revealed that nucleoli are dynamic structures composed of more than 700 different protein species. It is becoming evident that nucleoli contain multifunctional proteins that can play many essential roles in cellular

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### Abbreviations used

AAV, adeno-associated virus; AdV, adenovirus; Cap, capsid protein; CVB, Coxsackie B virus; DBP, DNA binding protein; HCMV, human cytomegalovirus; HDAG, HDV-specific antigen; HIC, human I-mfa domain-containing protein; HIPK, homeodomain-interacting protein kinase; HPIV, human parainfluenza virus; HPV, human papilloma virus; IRES, internal ribosome entry site; KSHV, Kaposi's sarcoma-associated herpes virus; NPM, nucleophosmin; NS5B, non-structural 5B protein; Nup, nucleoporin; PKR, double-stranded RNA-dependent protein kinase; Pol, polymerase; P-TEFb, positive transcription elongation factor b; rDNA, ribosomal DNA; DNA sequence coding for the 45S ribosomal RNA precursor; Rep, replication protein; RRE, rev responsive element; rRNA, ribosomal RNA; SARS-CoV, severe acute respiratory syndrome coronavirus; SL-1, RNA Pol I selectivity factor; TAR, Tat transactivation responsive region; UBF, RNA Pol I upstream binding factor.



**Figure 1.** Virally induced modifications of nucleoli of cells infected with a wild-type strain of HSV-1. **A**-Optical microscope images of HeLa cells infected for the indicated periods of times, in hours. The arrows show nucleoli. The morphology of nucleoli is modified during the course of infection, although they remain visible even at late times of infection. **B**-Confocal laser microscope images, after immunofluorescence analysis of nucleolin in red and B23 in green. Nucleolin and B23 co-localise in the nucleoli of non-infected cells, but are delocalised in different cell areas as revealed by their partial co-localisation in the merge image from cells infected for 15 h.

pathways, in addition to ribosome biogenesis, and that most of these proteins are in constant flux with the other cell compartments in response to various perturbations, including stress, cellular diseases and viral infections [2–7].

The nucleolar structural integrity depends on the RNA polymerase I (Pol I) activity and on the correct expression of the nucleolar protein nucleolin/C23 [8]. The two most abundant nucleolar proteins, nucleolin and B23 also called nucleophosmin (NPM), are involved in ribosome biogenesis and in many other functions that depend on their post-translational modifications status. Nucleolin is a ubiquitous, highly phosphorylated and mobile protein, which participates in many essential roles, such as chromatin remodelling, DNA recombination and replication, RNA transcription by RNA Pol I and II, rRNA processing, mRNA stabilisation, cytokinesis and apoptosis (for reviews see References [9,10]). Nucleolin promotes cell proliferation [11–13] whereas nucleolin depletion promotes cell cycle arrest and apoptosis [8]. B23 is involved in nuclear/cytoplasmic trafficking, and association with B23 directs many proteins to nucleoli. B23 regulates DNA polymerase activity and centrosome duplication, and possesses chaperone activities [11–14].

Replication of viruses with a DNA or a RNA genome generally takes place in the nucleus or in the cytoplasm, respectively. During infection of cells with human viruses of various types, nucleoli undergo important morphological modifications

(see part A of the Figure 1), and numerous viral components traffic to and from the nucleolus where they interact with different cellular and/or viral factors. In addition, numerous host nucleolar proteins are redistributed to other cell compartments (see part B of the Figure 1 which shows the delocalisation of nucleolin and B23 nucleolar proteins upon HSV-1 infection) or are modified during infection, and some non-nucleolar cellular proteins are relocalised in the nucleolus of infected cells. At present, the roles of these virally induced nucleolar perturbations on viral replication and host cell functions are not fully elucidated. Nevertheless, several well-documented studies allow us to establish that nucleolar modifications play a role in some steps of the viral cycle, from viral entry to virus assembly and egress, including intracellular trafficking and also to fundamental cellular processes, such as transcription and regulation of the cell cycle.

#### EFFECT OF VIRALLY INDUCED NUCLEOLAR MODIFICATIONS ON VIRUS LIFE CYCLE

The viral and cellular proteins that are targeted to nucleoli during infection with human viruses, and the nucleolar proteins that are delocalised and/or that could interfere with the viral cycle are indicated in the Table 1. Usually, proteins that have nuclear/nucleolar trafficking ability possess sequences involved in their nuclear and nucleolar localisation [15–17].

**Table 1. Viral and cellular proteins that are targeted to nucleoli during infection with human viruses, and host nucleolar proteins delocalised and/or potentially interfering with the viral life or cellular pathways**

Virus	Involved nucleolar proteins	Proteins redistributed to nucleoli		Function	References
		Viral	Cellular		
<b>DNA viruses</b>					
AAV	Nucleolin, B23	Cap, Rep		Traffic, assembly	[49–52]
AdV	Nucleolin, B23, UBF, Fibrillarin, RNA Pol I	Core V, preVII, preMu, IVa2, UXP, E4orf4		Traffic, replication	[53,74–79,91,117–120]
EBV	EBP2, p14ARF, Fte-1	EBNA-5	hsp70		[121–124]
HBV		Capsid		Cell cycle	[104]
HCMV		TRL-5, TRL-7, TRL-9, UL27, UL29, UL31, UL83, UL108, US33			[125–127]
HPV	Nucleolin			Transcription	[68,69]
HSV-1	Nucleolin, B23, UBF, Fibrillarin	ICP0, ICP4, ICP27, gamma34.5, UL24, UL27.5, Us11, VP22	cell cycle	Traffic, replication,	[54,80,81,105,127–136]
HSV-2		UL3, UL24, UL31, Us11			[116,137–139]
KSHV			Angiogenin	Host transcription	[88,89]
<b>RNA viruses</b>					
CVB	Surface nucleolin			Entry	[26]
HCV	Nucleolin, B23, RNA Pol I, UBF, SL1, TBP, p53	Core, NS5B	PKR	Traffic, translation, replication, host transcription, cell cycle	[57–61,72,73,82,92,93]
HDV	Nucleolin, B23, SL-1	HDAg		Traffic, replication	[55,56,83–85]
HIV	Nucleolin, B23, cdk9, NFBP	Rev, Tat	CRM1, granulins, HIC, Nup98, Nup214, 16.4.1	Entry, traffic, transcription, budding	[19,20,22–24,27–32,35–40,63,64,86,140]
HPIV	Surface nucleolin			Entry	[25]
HTLV	B23	Rex, p30, HBZ-SP1	CRM1	Traffic	[41–43,45–47,141]
Influenza	Nucleolin	NS1A			[142,143]
Poliovirus	Nucleolin, UBF, SL-1			Translation, host transcription	[70,71,94]
SARS-CoV	B23	N protein, 3b		Cell cycle	[96–100,102,103,144]

### Viral attachment and entry

The efficient viral entry into the host cell is facilitated by the interaction of viral proteins with host proteins. Nucleolin, besides its main nucleolar localisation, is also present at the surface of some types of cells, driving proliferation [18]. Cell surface-expressed nucleolin can act as a co-receptor for the entry of several viruses. Indeed, stimulated lymphocytes from HIV-infected patients show abnormal nucleolar structure and an increase in nucleolin membrane localisation [19–21]. Surface nucleolin behaves like an HIV receptor. Anchorage of HIV particles induces nucleolin aggregation, and HIV attachment to cells is almost totally prevented in cells pre-incubated either with a pseudo-peptide mimicking a region of viral gp120 protein essential for receptor binding and cell attachment, or with a peptide corresponding to the C-terminal region of nucleolin. This indicates that surface nucleolin is implicated in virus attachment probably through interaction with gp120 [22–24]. After HIV entry, the amount of cell surface nucleolin decreases due to its translocation into the cytoplasm where the amount of nucleolin increases [22].

Cell-surface nucleolin has been identified also at the surface of human lung epithelial cells. It interacts with human parainfluenza virus type 3 (HPIV-3) envelope proteins, leading to efficient viral entry. HPIV-3 infection and virus internalisation are inhibited by nucleolin antibodies, indicating that cell-surface nucleolin is involved in the internalisation process. However, data suggest that HPIV-3 entry requires in addition another unidentified molecule [25].

Surface nucleolin, present in Coxsackie B virus (CVB)-permissive human cells, is a specific binding protein for CVB, suggesting that it could be a putative CBV receptor [26].

### Trafficking

The role of nucleolar trafficking in viral infection is especially well documented in the case of HIV. The major HIV regulatory proteins, Tat and Rev, are prominent within the nucleolus of infected cells, and B23 plays a critical role in their nucleolar localisation [27–30]. Tat transactivates proviral DNA transcription. Interaction of B23 with Tat is necessary for the nucleolar localisation of Tat [29], and Tat expression induces the redistribution in the nucleolus of human I-mfa domain-contain-

ing protein (HIC) and granulins [31,32]—two proteins that modulate Tat activity.

Rev promotes the nuclear export of singly spliced and unspliced viral RNAs by binding to the Rev responsive element (RRE) present in some viral RNAs. For this, Rev has to shuttle between the cytoplasm and the nucleus, to bind viral RNAs and to interact with various nuclear import and export factors. The nucleolus plays a central role in this process. B23 imports Rev into the nucleus and the nucleolus [27,28]. A series of reports demonstrate that HIV RNA passes through the nucleolus before nuclear export [33,34], and that the nucleolar trafficking of Rev and of viral RNA is critical for the outcome of infection. Indeed, sequestration of Rev in the nucleolus impairs HIV-1 infection [35], and HIV-1 infection is prevented in cells expressing a nucleolar-localised hammerhead ribozyme that specifically targets the viral RNA [34]. The efficient export of Rev-RNA complexes requires the multimerisation of Rev and the interaction of Rev with the cellular export factors CRM1, nucleoporin 98 (Nup98) and Nup214. It has been shown that Rev multimerises predominantly in nucleoli [36], and that expression of Rev leads to nucleolar targeting of CRM1, Nup98 and Nup214 [37–39]. Therefore, it has been speculated that complexes containing Rev, CRM1, Nup98 and Nup214, could assemble in the nucleoli or in the nucleoplasm, and export Rev-RNA from the nucleus to the cytoplasm [39]. Rev recruits also 16.4.1 protein in the nucleolus, a cellular modulator of Rev activity. However, the physiological role of this interaction is not clear [40].

The nucleolus also plays a central role in the nuclear export of mRNAs encoding the structural and enzymatic proteins of the HTLV. HTLV Rex protein, in association with CRM1, activates viral mRNA export, while viral protein p30 is suspected to prevent it [41–43]. Both Rex and p30 proteins are found in the nucleolus [44–46]. The nucleolar import of Rex seems to be mediated by B23, and relies also on Rex phosphorylation status [47,48]. A recent study shows that Rex recruits CRM1 in nucleoli, and that CRM1 is present in all the sub-nuclear structures containing Rex and also in nuclear bodies containing the viral transactivation factor Tax. It also reveals that p30 interacts with Rex, and over expression of p30 results in the sequestration of Rex/CRM1 complexes in the

nucleoli [45]. It was therefore proposed that nucleoli could be the route of entry of Rex in the nucleus and the site where Rex and CRM1 assembled complexes are sequestered by the negative regulator p30 [45]. Assembly of Rex/CRM1 complexes with their target RNAs would occur after redistribution of these complexes to the nucleoplasm in order to favour their export to the cytoplasm.

Adeno-associated viruses (AAVs) need a helper virus to initiate their viral cycle. In cells co-infected with AAV and adenovirus (AdV), nucleoli increase in size and viral capsid (Cap) and replication (Rep) proteins are localised in nucleoli [49–51]. The relevance of Cap/Rep nucleolar targeting is developed in a following paragraph. Both viral proteins bind to nucleolin and B23 [50,51]. In a very recent report, recombinant AAV2 (rAAV2) vectors were tracked in order to determine the cellular conditions that control AAV Cap trafficking. After infection, rAAV2 virions enter the nucleus, accumulate in the nucleolus where they retain their infectivity, and remain sequestered there in a stable form [52]. Knockdown of B23 potentiates nucleolar accumulation, while knockdown of nucleolin mobilises Caps to the nucleoplasm, and viral transduction is improved in both cases [52]. In light of the helper-dependent nature of AAV, it was speculated that AAV would utilise nucleolar proteins for sequestration of incoming virus particles in nucleoli and would exploit nucleolar disruption (mitosis, coinfection, etc.) to trigger genome release under favourable conditions [52].

It has been described that, upon infection with other human viruses, many viral or cellular components transit through nucleoli. On the other hand, host nucleolar proteins leave nucleoli (see Table 1). However, the functional significance of these modifications is not yet elucidated. Some of them are involved in the redistribution of other proteins. This is the case for the AdV core protein V that is targeted to the nucleolus, and that induces the redistribution of nucleolin and B23 to the cytoplasm when expressed on its own [53]. In cells infected with HSV-1, UL24, which is one of the numerous viral proteins that localise in the nucleolus (see Table 1), have the ability to redistribute nucleolin out of nucleoli [54]. Nucleolin interacts with HDV-specific antigens (HDAg) and this binding is required for targeting HDAg to nucleoli

[55,56]. Nucleolin interacts also directly with HCV non-structural 5B (NS5B) protein and this interaction is responsible for the redistribution of nucleolin in the cytoplasm [57,58]. In addition, the expression of HCV core protein, which was detected in the nucleolus of hepatocytes from a chronically HCV-infected patient, facilitates translocation of PKR into nucleoli [59–61].

### Transcription

The nucleolar targeting of HIV Tat protein involved in proviral DNA transcription is critical for viral replication. During HIV infection, the viral Tat protein is essential for regulating viral transcription initiation complex assembly. B23 targets Tat to the nucleolus, and Tat recruits the positive transcription elongation factor b (P-TEFb), its main critical cellular co-factor, to the Tat transactivation responsive region (TAR) element on HIV RNAs [62]. The P-TEFb catalytic subunit is composed of cdk9/cyclin T1. Protein localisation studies show that several cellular factors that modulate Tat activity are either present in the nucleolus, i.e. cdk9 and NFBP, or are relocalised in nucleoli when Tat is expressed, i.e. HIC and granulin [31,32,63,64]. In addition, interaction of Tat with cyclin T1 has been visualised in the nucleolus [65]. It has been shown that nucleolar trafficking of Tat is critical for HIV-1 infection. Indeed, the expression of a chimeric small nucleolar-localised RNA-TAR element potentially inhibits HIV replication [66,67]. Altogether, these results strongly suggest that these proteins participate in the formation of multimolecular complexes governing coordinated steps of HIV proviral DNA transcription, and that the nucleolus plays an essential role in the modulation of this proviral DNA transcription.

In human papillomavirus (HPV)-induced cancer cells, nucleolin shows an abnormal granular intranuclear distribution [68]. In cervical cancer, nucleolin specifically binds to the enhancer of E6 and E7 oncogenes encoded by HPV-18 and activates transcription in S phase of the cell cycle. Indeed, nucleolin controls the formation of a DNase I hypersensitive site in the HPV-18 enhancer leading to an open chromatin structure. This suggests that nucleolin facilitates the binding of other transcription factors in E6/E7 oncogene enhancer, and therefore controls the proliferation of HPV-18 cervical cancer cells [68,69].



## Translation

Nucleolin regulates viral protein synthesis in cells infected with poliovirus and HCV, two viruses that use internal ribosome entry site (IRES)-mediated translation for their mRNA. Infection of cells with poliovirus results in the redistribution of nucleolin into the cytoplasm [70]. Data reveal that nucleolin binds to the viral RNA, and stimulates its IRES-mediated translation. Nucleolin could act as a chaperone in the preinitiation complex formation to favour viral mRNA translation over that of the cellular capped-mRNAs [71].

In the case of HCV, nucleolin has been identified as an HCV-IRES-binding protein in two independent studies applied to determine the bulk of proteins interacting with the IRES of viral mRNA [72,73]. This suggests a possible function for nucleolin during HCV IRES mediated mRNA translation.

## Replication

Several host nucleolar proteins play a critical role in replication of the genome of different viruses. In AdV-infected cells, viral UXP protein that seems to be required for the establishment of the viral replication centres passes through nucleoli [74]. In addition, three host nucleolar proteins—B23, RNA pol I and its upstream binding factor (UBF)—relocalise at least in part to the viral replication centres, and B23 and UBF participate in viral DNA replication [75–77]. Indeed, UBF co localises with regions of newly replicated viral DNA and appears associated with the ends of the viral genome, and, when recruited into the viral replication centres, UBF enhances viral DNA replication [77]. On the another hand, B23 stimulates AdV DNA replication *in vitro* [76,77], and interacts with viral core proteins V and pre-VII in infected cells to function as a chaperone in the viral chromatin assembly process [78]. Recent data demonstrate also that both B23.1 and B23.2 isoforms regulate viral DNA replication through differential interactions with the two viral replication proteins pTP and DBP suggesting that both B23 isoforms are involved in different steps of the viral DNA replication process [79].

Upon HSV-1 infection, and at the time when HSV-1 replication is active, nucleolin and UBF are redistributed to the viral replication compartments where they are co-localised with viral ICP8 protein, a DBP protein essential for viral gen-

ome replication, and a marker of viral replication compartments [80,81]. Viral replication compartments are the exclusive sites of replication, transcription and encapsidation of HSV-1 genomes. B23 seems also to be present in viral replication compartments [80]. The role played by these nucleolar proteins in the viral replication compartments has not yet been fully elucidated. However, UBF is redistributed into viral replication compartments of infected cells, and participates in viral DNA replication [81]. Nucleolin is necessary for the outcome of infection, since in nucleolin knock-down cells, both viral gene expression and virus yields are decreased, while viral adsorption to host cells is not inhibited [80]. This suggests that nucleolin may play a role in viral DNA replication.

In HCV-infected cells, viral RNA replication occurs in the cytoplasm. It has been shown that nucleolin is translocated from the nucleolus to the cytoplasm of infected cells, and that nucleolin redistribution is induced by its interaction with the viral NS5B protein, which is the viral RNA-dependent RNA polymerase and the central catalytic enzyme in HCV replication [57,58]. One of the NS5B regions involved in the interaction with nucleolin is essential for oligomerisation of NS5B. This is a prerequisite for the RNA-dependent RNA polymerase activity. In addition, virus yields are reduced in nucleolin knockdown cells. This indicates that nucleolin regulates HCV RNA replication, probably through its interaction with NS5B [82].

The nucleolus is also involved in the replication of the HDV genome which is a single-strand circular RNA of negative polarity, that encodes a single protein, the delta antigen HDAg, expressed in two forms, small and large HDAg. Small HDAg is required for HDV RNA replication. Both isoforms have nucleolar localisation, and their interaction with nucleolin contribute to HDAg nucleolar localisation [55,56]. B23 interacts also with HDAGs and modulates viral RNA replication [83]. Recent data reveal that HDV antigenomic RNA synthesis from the genomic RNA template occurs in the nucleolus, and is carried out by RNA Pol I or a Pol I-like protein and involves the RNA Pol I-specific selectivity transcription factor SL-1 [84,85]. Indeed, SL-1 interacts with HDAG, and pre-treatment of nuclear extracts with an anti-SL-1 antibody drastically reduces HDV antigenomic RNA synthesis *in vitro* [84].

Therefore, viruses divert several host nucleolar proteins to promote their own genome replication.

### Virus assembly and egress

A role for B23 in the assembly of AAV Caps has been well documented recently, even if it has been known for many years that AAV Cap assembly takes place in nucleoli of AAV-AdV co-infected cells [49–51]. Indeed, Cap proteins first accumulate in the nucleoli, then spread over the whole nucleoplasm. Expression of the Cap gene alone leads to the formation of capsids which are restricted to the nucleoli, while upon coexpression of Rep, Cap proteins leave the nucleolus, suggesting a role for Rep in capsid export [49–51]. B23 protein, in addition to its ability to interact with AAV Rep proteins, also stimulates the interactions of Rep with the AAV genome [51]. A model has been proposed, in which Cap proteins accumulate and assemble in the nucleolus where they associate with B23 and perhaps other nucleolar proteins, and AAV Rep proteins favour the exit of complexes containing Rep, B23 and Cap proteins from the nucleolus to the nucleoplasm where viral DNA encapsidation occurs [49–51].

It has been shown that nucleolin and HIV gag proteins form a complex incorporated into virions, suggesting that nucleolin might be involved in the transfer of gag proteins to the membrane, and to the release of virions. In addition, nucleolin promotes the infectivity of HIV-1 [86].

### CONSEQUENCES FOR CELLULAR PATHWAYS

#### Cellular transcription

The virally induced nucleolar modifications could interfere with host DNA transcription mediated by RNA Pol I or Pol II. The accurate initiation of transcription from the DNA promoter of the 45S rRNA precursor gene, i.e. rDNA, requires RNA Pol I, and UBF and SL-1 specific RNA Pol I factors [87].

Infection of sub-confluent cells with Kaposi's sarcoma-associated herpes virus (KSHV) induces angiogenin to move into the nucleolus. Angiogenin binds to elements involved in rDNA transcription, and increases transcription from the rDNA promoter [88,89]. This suggests that nucleolar angiogenin stimulates rDNA transcription, and this could play a role in KSHV induced anti-apoptosis [89].

Upon AdV infection the synthesis and export of rRNAs are blocked [90]. However, although several nucleolar proteins involved in ribosome production are delocalised out of nucleoli of infected cells, i.e. RNA Pol I, nucleolin, B23, fibrillarin, and UBF [53,75,77,91], until now none of them has been shown to be responsible of this dysfunction. Indeed, data reveal that the delocalisation of the majority of UBF out of the nucleolus does not suppress rRNA synthesis [77].

HCV core protein regulates different transcriptional pathways in the host cells. It activates transcription by RNA Pol I. Indeed, core protein associates with SL-1 via direct contact with the TATA-binding protein, and enhances the recruitment of RNA Pol I and UBF to the rDNA promoter. This is accompanied by a specific hyperphosphorylation of UBF [92]. HCV core also activates RNA Pol II-mediated transcription. It has been shown that core protein binds B23 and p300, orchestrates the formation of a large transcriptional complex on the B23 promoter, and activates B23 expression [93]. These findings support an active role of core protein in promoting cell growth, proliferation, and the progression of liver carcinogenesis during HCV infection.

In poliovirus-infected cells, rRNA synthesis is inhibited and both SL-1 and UBF nucleolar transcription factors are modified and inactivated, SL-1 being cleaved by the viral protease 3C [70,94]. This virally induced depletion of SL-1 and UBF is suspected to contribute to the inhibition of RNA Pol I-mediated transcription since addition of these two purified factors restores rDNA transcription *in vitro* [94].

#### Cell cycle regulation and apoptosis

Viral replication can change the rate of proliferation of susceptible cells. One example comes from the severe acute respiratory syndrome coronavirus (SARS-CoV) encoded N protein, the nucleolar localisation of which is related to cell cycle regulation [95–98]. It has been shown that viral N protein can arrest cell cycle progression, and inhibit the phosphorylation of B23 on Thr199 [99,100]. B23 protein has the ability to specifically associate with unduplicated centrosomes. However, when B23 is phosphorylated on Thr199 it dissociates from centrosomes, which consequently initiate their duplication [101]. A model has been proposed where the interaction of N protein with

B23 results in the lack of B23 protein phosphorylation on Thr199, leading to cell cycle arrest since the centrosomes cannot initiate duplication [100]. The expression of the other viral nucleolar protein, 3b, induces necrosis and apoptosis. However, the 3b nucleolar localisation does not seem to be important for the cell-death pathways [102,103].

HCV core protein, that has been shown to interact with p53 and to facilitate translocation of PKR into nucleoli where the two proteins co-localise, is suspected to induce PKR-dependent apoptosis [60,61].

The HBV Cap protein is localised in nucleoli of a limited number of cells, and those cells are often bi-nucleated or apoptotic, suggesting that Cap proteins present in the nucleolus may perturb cytokinesis [104].

Finally, the HSV-1 nucleolar Us11 protein interacts with the homeodomain-interacting protein kinase 2 (HIPK2), a protein that plays a role in p53-mediated cellular apoptosis and could also participate in the regulation of the cell cycle. Us11 also modifies the sub-cellular distribution of HIPK2 and protects the cell against the HIPK2-induced cell growth arrest [105].

## DISCUSSION

The efficiency of viral infection in its host cells depends on the availability and the correct localisation of viral and cellular factors. In cells infected with human viruses, nucleoli undergo important modifications at the level of structure and composition. This is the case also after infection of cells with animal or plant viruses [6,17,106–110]. Several of these nucleolar modifications play a role in some steps of the viral cycle, and also in fundamental cellular pathways.

It appears that the nucleolus itself is the place where several essential processes take place. HDV subgenomic RNA synthesis occurs in the nucleoli of infected cells, and several host nucleolar proteins participate in this process. The nucleolus is the place where AAV capsids accumulate and assemble before their export from the nucleus, and it is also suspected to be the place where the incoming AAV particles are sequestered in an active form until viral genome release is triggered by favourable conditions. The nucleolus is also the place where factors that regulate the activity of HIV Rev and Tat proteins, and of HTLV Rex proteins, are already present or are redirected from

other cell compartments. This indicates that the nucleolus plays a central role in the regulation of the activities of these essential viral proteins, and consequently in the outcome of infection. In the case of HIV infection, targeting nucleoli is already a part of antiviral strategies. As demonstrated, HIV RNA, and Rev and Tat proteins traffic through the nucleolus, and it has been shown that targeting anti-HIV RNA to the nucleolus or sequestering Rev or Tat in the nucleolus reduces the efficiency of infection [35,66,67,111–113].

It is interesting to note that at least three viral proteins that target the nucleoli, i.e. HIV Tat, HSV-1 VP22 and HSV-2 Us11, are able to spread from cell to cell during infection [114–116]. This intercellular trafficking activity probably contributes to their distribution.

Viral or host proteins that are redistributed to nucleoli upon infection with KSHV, HCV or poliovirus participate in the virally induced positive or negative regulation of rDNA transcription.

Some viruses divert nucleolar functions by redirecting specific host nucleolar proteins from the nucleolus to different cell compartments where they play essential role(s) in the virus life cycle. Nucleolin redistributed or enhanced at the cell surface is involved in HIV and HPIV, and probably CVB entry process. Nucleolin is translocated to the cytoplasm of HCV and poliovirus infected cells. Cytoplasmic nucleolin participates in the IRES-mediated translation of HCV and poliovirus mRNAs, and in the replication of the HCV genome. In the case of AdV and HSV-1 infection, nucleolin, B23 and UBF nucleolar proteins are redistributed to the viral replication compartments in the nucleus where they could participate in viral genome metabolism. In addition, SL-1 participates in the HDV antigenomic RNA synthesis that occurs in the nucleolus of infected cells. While the involvement of nucleolin and B23 in DNA metabolism is already well documented, the known role of UBF and SL-1 nucleolar factors is related to rDNA transcription. Therefore, these findings reveal that viruses divert some host proteins, including nucleolar proteins, from their conventional functions in order to exert new unexpected role(s).

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