ABSTRACT

The nucleolus is a nuclear domain involved in the biogenesis of ribosomes, as well as in many other important cellular regulatory activities, such as cell cycle control and mRNA processing. Many viruses, including herpesviruses, are known to exploit the nucleolar compartment during their replication cycle. In a previous study, we demonstrated the preferential targeting and accumulation of the human cytomegalovirus (HCMV) UL83 phosphoprotein (pp65) to the nucleolar compartment and, in particular, to the nucleolar matrix of lytically infected fibroblasts; such targeting was already evident at very early times after infection. Here we have investigated the possible effects of rRNA synthesis inhibition upon the development of HCMV lytic infection, by using either actinomycin D or cisplatin at low concentrations, that are known to selectively inhibit RNA polymerase I activity, whilst leaving RNA polymerase II function unaffected. Following the inhibition of rRNA synthesis by either of the agents used, we observed a significant redistribution of nucleolar proteins within the nucleoplasm and a simultaneous depletion of viral pp65 from the nucleolus; this effect was highly evident in both unextracted cells and in nuclear matrices in situ. Of particular interest, even a brief suppression of rRNA synthesis resulted in a very strong inhibition of the progression of HCMV infection, as was concluded from the absence of accumulation of HCMV major immediate-early proteins within the nucleus of infected cells. These data suggest that a functional relationship might exist between rRNA synthesis, pp65 localization to the nucleolar matrix and the normal development of HCMV lytic infection. J. Cell. Biochem. 108: 415–423, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: HUMAN CYTOMEGALOVIRUS; NUCLEAR MATRIX; NUCLEOLUS; rRNA; ppUL83 (pp65); IEp72

The nucleolus is one of the most prominent compartments of the cell nucleus and the most extensively studied nuclear domain. It is organized around ribosomal DNA (rDNA) repeats, which cluster at chromosomal loci called nucleolar organizers, and it is the site of rRNA transcription, processing and assembly into ribosomal subunits. Recent studies have also highlighted that nucleoli possess non-canonical functions, being involved in the cell cycle regulation, cellular stress responses, apoptosis and viral replication [Pederson, 1998; Olson et al., 2000; Boisvert et al., 2007]. Considering these regulatory functions of the nucleolus, it is not surprising that animal viruses exploit this structure as part of their infection strategy. Many different viruses target their own proteins to the nucleolus and/or recruit nucleolar proteins, such as adenovirus, which interacts with nucleophosmin [Okuwaki et al., 2001], avian bronchitis coronavirus, whose nucleocapsid protein localizes to the nucleolus [Dove et al., 2006], *Herpes simplex virus* type 1 that induces dramatic modifications to nucleolar morphology and interacts with nucleolin during productive infection [Besse and Puvion-Dutilleul, 1996; Callé et al., 2008] and HIV-1 Tat and Rev proteins, thought to be involved in the export of unspliced viral mRNA out of the nucleolus [Cochrane et al., 1990; Siomi et al., 1990].

With regard to the relationships between HCMV and the nucleolus, it has been described that significant changes in the nucleolar morphology and a substantial enhancement of rRNA transcription occur at early stages in experimentally infected human
embryo fibroblasts [Jarskaja et al., 2002]. Moreover, a recent study by our own group demonstrated the nucleolar accumulation of the HCMV tegument protein pp65 following the infection of human fibroblasts in vitro; such spatial targeting of viral pp65 was found to be most prominent at early times after HCMV entry and was also evident within nuclear matrix-associated remnant nucleoli [Arcangeletti et al., 2003].

It is clear that a deeper knowledge of how the host transcription apparatus is redirected for viral gene expression will provide the key to reveal how HCMV infection develops within the host cell. Thus, focusing research on HCMV component compartmentalization within the nucleus and the nuclear matrix and unveiling the cellular processes that are potentially involved in the regulation of viral gene expression will greatly aid our understanding of what is able to interfere with the normal lytic cycle and likely contribute to the establishment of a latent condition.

In order to investigate further these important, yet poorly understood, features of HCMV–host cell interactions, this article addresses the effects of suppressing ribosomal gene transcription upon the relationships between nucleolar functions (such as rRNA synthesis), the nucleolar and nucleolar matrix targeting of viral pp65 and HCMV gene expression over the course of lytic infection in vitro.

Our data show that even a brief inhibition of rRNA synthesis significantly affects the normal development of the lytic program of HCMV, and simultaneously leads to a redistribution of nucleolar proteins within the nucleoplasm, as well as the nucleolar depletion of viral pp65; these effects were particularly evident in nuclear matrices in situ.

MATERIALS AND METHODS

CELL CULTURE

Monolayer cultures of MRC5 human embryo lung fibroblasts (American Type Culture Collection, ATCC; CCL-171) were grown in Earle’s modified Minimum Essential Medium (E-MEM), supplemented with 2 mM l-glutamine, 1% non-essential aminoacids, 1 mM sodium pyruvate, 10% fetal bovine serum (FBS) and antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin). Cell culture medium and supplements were from Invitrogen.

VIRUS INFECTION AND TITRATION

The reference AD169 strain (ATCC VR-538) of HCMV was used for the in vitro infection of MRC5 human embryo fibroblasts. Viral infectious titer was determined by plaque assay, as already described [Arcangeletti et al., 2003].

The AD169 strain was used at a multiplicity of infection (m.o.i.) of 1 plaque-forming unit (PFU)/cell.

DRUG TREATMENT

Stock solutions of 1 mg/ml actinomycin D (act-D) was prepared in dimethylsulfoxide (DMSO) and stored at −20 °C. Act-D was added to MRC5 cells 1 h 30 min before infection, at a final concentration of 0.05 μg/ml. Then, pre-treated monolayers were infected with AD169 strain at a m.o.i. of 1 PFU/cell and incubated at 37 °C for the planned times, in the presence of act-D. cis-Diaminedichloroplatinum (cisplatin or cis-DDP) was added to uninfected MRC5 fibroblasts for 7 h at a final concentration of 20 μg/ml (stock solutions of 20 mg/ml in DMSO, stored at −20 °C), then withdrawn before infection. Control cells were similarly incubated in the same dilution of DMSO as used for act-D and cis-DDP. Drugs and DMSO were purchased from Sigma–Aldrich.

IN SITU NUCLEAR MATRIX EXTRACTION

MRC5 fibroblasts were plated in 12 mm round glass cover-slips at low density (1.6 × 10⁵ cells/cell-slip) for 48 h, before they were treated with the drugs (or untreated) and infected with AD169 strain for the indicated times.

In situ nuclear matrices were obtained according to Arcangeletti et al. [2003]. Briefly, cell monolayers were rinsed twice with TM buffer [50 mM Tris–HCl (pH 7.5), 3 mM MgCl₂], and then incubated for 10 min on ice in TM buffer containing 0.5 mM phenylmethylsulphonyl fluoride (PMSF), 0.5% Triton X-100 and 0.5 mM CuCl₂. After washing with TM buffer supplemented with 0.5 mM PMSF, the cells were incubated at 37 °C for 20 min in TM buffer containing 100 units/ml RNase-free DNase I (Roche Diagnostics). The above buffer was then replaced by TM buffer supplemented with 2 M NaCl and 0.5 mM PMSF and incubated for 30 min on ice. The in situ nuclear matrices were washed twice with TM buffer and immediately stained to detect viral antigens by indirect immunofluorescence. Chemicals were from Sigma–Aldrich.

ANTIBODIES

The following primary antibodies were used: a purified monoclonal blend (clones 1C3 and AYM-1) reacting with the 65 kDa lower matrix structural phosphoprotein (pp65) of HCMV (Argene); a monoclonal antibody (Mab clone E13, Argene) specific for the common epitope encoded by exon 2 of the major immediate-early (IE) viral gene products (Iep72 and Iep86); the “ANA-N” serum, a human antiserum to nucleolar antigens (The Binding Site). Anti-pp65 and anti-IE Mabs were diluted 1:30 with 0.2% bovine serum albumin (BSA) in phosphate buffer saline [PBS: 7 mM Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.4, 137 mM NaCl, 2.7 mM KCl], while “ANA-N” serum was used undiluted. Alexa-Fluor tetramethyl-rhodamine-isothiocyanate (TRITC)-conjugated goat anti-mouse IgG and Alexa-Fluor fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG (H + L) (Molecular Probes) were used to detect viral antigens and nucleolar proteins, respectively, by immunofluorescence.

INDIRECT IMMUNOFLUORESCENCE

At the indicated time points after infection, MRC5 cells (and/or in situ nuclear matrices) were gently rinsed with cytoskeleton (CSK) buffer [10 mM Pipes (1,4-piperazinediethanesulfonic acid) pH 6.9, 100 mM NaCl, 1.5 mM MgCl₂, 300 mM sucrose], and simultaneously fixed and permeabilized with 2.5% Triton X-100 and 1% formaldehyde in CSK buffer at room temperature for 20 min [Arcangeletti et al., 1997].

The fixed fibroblasts were washed three times (5 min/wash) with PBS and unspecific, immunoreactive sites were saturated with 1% BSA (pH 8.0) in PBS for 10 min; then, the cells were incubated with primary antibodies for 1 h at 37 °C, in a humid chamber. After three
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Negative controls were examined in parallel to assess the specificity

of FITC and TRITC signals and the absence of any background.

No mutual cross-contamination between green and red signals

detected. Series of x–y sections were acquired, with a z-step of 0.38 μm from apex to base, to cover the whole height of the samples.

RNA ISOLATION AND RT–PCR ASSAY

Primary rRNA transcripts were examined by reverse PCR (RT–PCR), using primers (5′-AGACGCTAGCGGAAGG-3′ and 5′-AGGGCG-TGTCGGTGTTGC-3′) specific to the rRNA internal transcribed spacer 1 (ITS1), between the 18S and 5.8S genes, with a product length of 293 bp. Transcription of pre-mRNA was examined with primers specific for the third intron of the β-actin gene (5′-GTTCGCTACTTAATACAC-3′ and 5′-GCCCCCTCTACCGTTC-3′; product length: 242 bp). Total RNA was extracted according to the manufacturer’s instructions (Macherey-Nagel, NucleoSpin® RNAl). Integrity of RNA was examined by denaturing agarose gel electrophoresis and sample quality/quantity were estimated measuring its absorbance at 260 and 280 nm (Eppendorf, BioPhotometer). The template RNAs were reverse transcribed and subjected to PCR amplification using forward and reverse primers, as described above, according to SuperScript III One Step RT–PCR with Platinum Taq (Invitrogen). The thermal cycler was programmed so that cDNA synthesis was followed immediately by PCR amplification, as hereafter detailed: one cycle at 50°C for 30 min for cDNA synthesis; 25, 30, and 35 cycles (ITS1) or 30, 35, and 40 cycles (β-actin intron) of 30 s at 94°C (denaturation), 30 s at 62°C (annealing), and 30 s at 72°C (extension), then 5 min at 72°C. The amplification products were run on 2% agarose gel and ethidium bromide stained; molecular weight markers (1 kb Plus DNA ladder, from 100 bp to 1.2 kb) were from Invitrogen.

RESULTS

THE INHIBITION OF RIBOSOMAL RNA SYNTHESIS HAS A MODULATORY EFFECT ON HCMV IMMEDIATE–EARLY GENE EXPRESSION DURING THE INITIAL STAGES OF INFECTION

As previously demonstrated [Jordan et al., 1996; Jordan and Carmo-Fonseca, 1998; Verduin, 2006], low concentrations of act-D (0.05 μg/ml) and cis-DDP (20 μg/ml) are able to inhibit RNA polymerase I-mediated transcription, leaving RNA polymerase II (also necessary for virus gene transcription) and RNA polymerase III functions unaffected. In order to verify that these inhibitors exhibit similar effects in both non-infected and HCMV (AD169)-infected MRC5 human embryo fibroblasts, preliminary experiments were performed (Fig. 1). Total RNA was extracted from both uninfected and infected cells at 2 h 30 min and 4 h 30 min time points post-infection (p.i.), following their incubation in the presence and absence of each of the two inhibitors. The extracted RNAs were reverse transcribed and subjected to a semi-quantitative PCR amplification (RT–PCR) in order to reveal a 293 bp rRNA fragment, corresponding to the internal transcribed spacer 1 (ITS1) (Fig. 1A,A′), and a 242 bp sequence from the third intron of β-actin pre-mRNA (Fig. 1B,B′).

The results demonstrated that both act-D and cis-DDP significantly inhibit RNA transcription when used at the above-stated concentrations, while the transcription of β-actin mRNA remains unaffected.

Next, untreated and act-D– or cis-DDP–treated MRC5 fibroblasts were infected with HCMV AD169 for 2 h 30 min or 4 h 30 min and then stained with antibodies against immediate-early (IEp72 and IEp86) viral proteins (Fig. 2); nuclei were counterstained with DAPI. Already at 2 h 30 min p.i., an accumulation of IE proteins within the nuclei of untreated cells was clearly visible, while in the nucleolus of treated cells only a few, discrete, bright spots were detected (Fig. 2, panels a,c,e). Similar patterns were observed at 4 h 30 min p.i. (Fig. 2, panels b,d,f), although in some cases the fluorescence signal was slightly increased in act-D–treated cells (arrows in panel d). These results suggest that the suppression of nucleolar gene transcription somehow blocks the development of HCMV infection. Importantly, this short-term inhibition of rRNA transcription does not result in the depletion of ribosomes within the cytoplasmin, as demonstrated by a comparison of quantities of rRNA present in equivalent aliquots (see the GAPDH signal) of RNA extracted from uninfected and HCMV-infected control cells versus cells treated with rRNA transcription inhibitors (Fig. 3). The RNA samples were analyzed by electrophoresis, and the intensity of the bands was quantified by densitometric analysis; then, data obtained in four independent experiments were submitted to statistical analysis (two-way ANOVA) (not shown), demonstrating that there are no significant differences in the amount of 18S and 28S rRNA under the experimental conditions used to suppress rRNA transcription.

THE NUCLEOLAR AND NUCLEOLAR MATRIX TARGETING OF VIRAL pp65 ARE INHIBITED IN ACT-D– OR CIS-DDP–TREATED MRC5 FIBROBLASTS AT EARLY TIME POINTS AFTER INFECTION

As previously published [Arcangeletti et al., 2003], the tegument protein pp65 of the incoming HCMV is preferentially targeted to the
nucleoli of infected cells very soon after infection. Figure 4 shows confocal microscopy images of double-labeled (nucleolar proteins: green; pp65: red) MRC5 fibroblasts after 45 min, 1 h 30 min and 2 h 30 min p.i., in the absence of rRNA transcription inhibitors (Fig. 4, panels a–c; a’–c’) and following treatment with either act-D (Fig. 4, panels d–f; d’–f’) or with cis-DDP (Fig. 4, panels g–i; g’–i’). A drug-induced redistribution of nucleolar proteins was observed (Fig. 4, panels d–f; g–i). It was a gradual process and the consequences of cell treatment with either act-D or cis-DDP were not exactly the same. In cells treated with act-D, although a significant level of nucleoplasmic redistribution of nucleolar proteins was observed, the nucleoli were still detectable even at 2 h 30 min p.i. (i.e., after 4 h treatment with act-D, since the cells had been pre-treated with the drug for 1 h 30 min before infection [see Materials and Methods Section for details]) (Fig. 4, panel f). Different kinetics was observed for viral pp65: in cells treated with act-D, the viral protein was already fully displaced from the nucleolar compartment at 45 min p.i. (i.e., 2 h 15 min after the start of act-D treatment). In cells pre-treated for 7 h with cis-DDP, most of the nucleolar proteins were already distributed throughout the entire nuclear space or otherwise displaced to the perinucleolar compartment at 45 min p.i. (Fig. 4, panel g). Viral pp65 was similarly distributed at 45 min p.i. (Fig. 4, panel g’).

In connection with the above results, it should be outlined that nucleoli were visualized using polyclonal serum raised against a mixture of nucleolar antigens. Different nucleolar proteins are likely to show different affinities for the nucleolus (in particular, those related to rRNA synthesis); moreover, their presence within this compartment may be differentially affected by the inhibition of rRNA transcription. Thus, it is possible, that specific nucleolar proteins have the same displacement kinetics as pp65 following treatment with act-D.

In the next set of experiments, cells were extracted in order to obtain the in situ nuclear matrices and the residual nucleoli were inspected in the absence of HCMV, as well as at different times after infection in control cells and cells treated with either act-D or cis-DDP (Fig. 5). In this case the time course kinetics has been shortened compared to that of unextracted nuclei, in order to analyze events occurring at the initial stages of HCMV infection. The nucleolar matrix was still detectable in cells pre-treated with act-D or cis-DDP before the beginning of virus infection (Fig. 5, panels d,g), although significant redistribution of nucleolar proteins into nucleoplasm was quite evident (compare panels a,d,g in Fig. 5). A similar pattern of nucleolar proteins staining was observed at 30 min p.i. (Fig. 5, panels e,h). At this time point, pp65 colocalized with remnant nucleoli only in some of the infected and treated cells (white arrows,
in panels e,e'; h,h'). At 45 min p.i. remnant nucleoli were practically absent in nuclear matrices of treated cells (Fig. 5, panels f,i). Nevertheless, in several cases, a perinucleolar ring was still detectable; a similar pattern was observed for pp65 (Fig. 5, panels f,o,i,o). Based on these results, we concluded that the existence of the nucleolar matrix drastically depends on the ongoing transcription of ribosomal genes. Furthermore, our results show that pp65 is initially targeted to and then released from the nucleolar matrix, before its complete disassembly.

**DISCUSSION**

The importance of nuclear compartmentalization for the regulation of gene expression is becoming increasingly evident [Pliss et al., 2005; Gorski et al., 2006; Malyavantham et al., 2008; Misteli, 2008]. One of the best characterized nuclear compartments is the nucleolus, which is presently considered not only as "the ribosome factory," but also as a multifunctional area that is involved in several cellular processes, including mRNA maturation and shuttling to cytoplasmic sites, control of the cell cycle, cell proliferation and apoptosis [Pederson, 1998; Verdun, 2006]. It is also worth mentioning that some important nucleolar proteins with known DNA and/or RNA binding properties and regulatory functions in rRNA synthesis and cell cycle control, like nucleophosmin (B23) and nucleolin (C23) [Grinstein et al., 2006; Ugrinova et al., 2007], have also been found to associate with the nuclear matrix; thus, they might be able to affect the whole system of nuclear compartmentalization [Mittnacht...
The nucleolus is frequently targeted by different DNA and RNA viruses during lytic infection; virus-specific proteins often accumulate in nucleoli or form complexes with nucleolar antigens [Miyazaki et al., 1995; Besse and Puvion-Dutilleul, 1996; Matthews, 2000; Pokrovskaja et al., 2001; Chen et al., 2002; Hiscox, 2002; Boyne and Whitehouse, 2006; Michienzi et al., 2006; Shimakami et al., 2006; Calle et al., 2008]. Another scenario is the redistribution of nucleolar proteins within the nucleoplasm or cytoplasm, caused by viral infection [Matthews, 2000; Bevington et al., 2007; Calle et al., 2008].

In this article, we show that early nucleolar compartmentalization of the major tegument protein ppUL83 (pp65) of the infecting HCMV [as previously described by Arcangeletti et al., 2003] is rapidly lost, or does not even become established, following the blockade of rRNA synthesis and delocalization of nucleolar proteins. Most importantly, we have demonstrated that rRNA synthesis blockade interferes with the development of HCMV lytic infection and that the latter is likely to stop at a very early stage, when the new formed IEp72 immediate-early protein is targeted to PML-bodies [Ahn and Hayward, 1997; Ishov et al., 1997; Arcangeletti et al., 2003]. Indeed, in cells treated with inhibitors of rRNA transcription, IE proteins are visible in a few speckles, possibly concomitant to colocalization with PML-bodies (Fig. 2), but further accumulation of this virus-specific protein is blocked. It is important to note that a similar effect was observed when act-D or cis-DDP, that is, agents affecting nucleolar gene transcription by different mechanisms were used (act-D preferentially intercalates with GC-rich DNA sequences; cis-DDP directly interferes with the Upstream-Binding Factor). Taking into account the different nature of these drugs, it is highly unlikely that the observed inhibitory effects are due to their direct action on HCMV. It is also worth mentioning that the lack of IE proteins accumulation cannot be due to the shortage of ribosomes necessary for the synthesis of virus-specific proteins as the effect is observed after a relatively brief time of cell cultivation under conditions of rRNA transcription suppression. Furthermore, we present evidence indicating that this short-time inhibition of rRNA transcription does not result in an overall decrease of mature rRNA present in the cells (Fig. 3). Thus, it appears evident that a regulatory pathway exists that blocks the progression of HCMV infection following the inhibition of rRNA synthesis. To this end, it is worth mentioning that many viruses are able to affect the activity of nucleolar genes through different mechanisms. For example, it has been reported that the HIV Tat protein localizes to nucleoli and interacts with fibrillarin, leading to an impairment of rRNA maturation, as well as a significant decrease in the number of cytoplasmic ribosomes, and directs the cell toward apoptosis [Ponti et al., 2008]. In addition, poliovirus induces rRNA synthesis shut-off [Banerjee et al., 2005], whereas adenovirus infection leads to nucleolar dysfunction and disruption of rDNA processing and transport to the cytoplasm [Lawrence et al., 2006]. As for herpesviruses, infection by Herpes simplex virus type 1 was shown to induce an early cellular protein synthesis shut-off, while the synthesis of rRNA and ribosomal proteins, as well as ribosome assembly, proceeded until late time points after infection. Nevertheless, ribosomes show several virus-induced modifications, including unusual patterns of phosphorylation and association between cellular and viral proteins [Simonin et al., 1997; Diaz et al., 2002].

Unlike other herpesviruses, HCMV is able to stimulate host cell RNA synthesis, including ribosomal RNA [Tanaka et al., 1975; Jarskaja et al., 2002], and this is accompanied by a profound reorganization of the nucleolus [Jarskaja et al., 2002]. Therefore, it is likely that the presence of HCMV components within the nucleolar...
compartment is important for the virus in order to redirect some nucleolar functions for its own benefit. Although the function(s) of pp65 remain(s) to be fully understood, there are many observations suggesting that this protein is involved in the regulation of the initial events of viral replication. First of all, this hypothesis is supported by the previous observations made by ourselves and other groups regarding the nucleolar and nuclear matrix localization of the incoming and the de novo synthesized pp65 [Sanchez et al., 1998; Arcangeletti et al., 2003]. Furthermore, it has been reported that pp65 has a kinase activity [Britt and Auger, 1986; Somogyi et al., 1990] and interacts with other viral and/or cellular kinases [Gallina et al., 1999; Kamil and Coen, 2007]. Finally, Dal Monte and collaborators found that HCMV replication in vitro is suppressed in the absence of a functional pp65 [Dal Monte et al., 1996].

The present data reinforce the hypothesis postulating an involvement of pp65 viral tegument protein in regulatory/signaling pathways and suggest that the latter are linked to the function of the nucleolus and depend (directly or indirectly) on rDNA transcription. In this respect it might be important that control of the cell cycle of mammalian cells is connected to the biogenesis of ribosomes at the nucleolar level [Ruggero and Pandolfi, 2003; Pliss et al., 2005]. During G1, an increase in rRNA synthesis and ribosome assembly are necessary to satisfy an increased need of newly synthesized proteins at the beginning of the S phase. On the other hand, it is known that HCMV is able to modify the normal progression of the cell cycle to its own benefit, by blocking cycling cells in G1 and G1/S [Bain and Sinclair, 2007]. Nucleolar components control the above-mentioned phases, mostly by modulating phosphorylation levels of transcription factors that can influence their interaction with and activation of polymerase I transcription machinery [Russell and Zomerdijk, 2005]. Thus, it is likely that the targeting to the nucleolus of the incoming pp65, possessing a kinase activity, represents a crucial strategy adopted by HCMV to take over the regulatory systems controlling the cell cycle progression in order to ensure the correct development of the lytic program.

Work is currently in progress to identify the nucleolar partner(s) of pp65 and to test the possible relationships between this viral protein, the nucleolus, the rRNA synthesis and the regulation of HCMV gene expression.

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REFERENCES

Ahn JH, Hayward GS. 1997. The major immediate-early proteins IE1 and IE2 of human cytomegalovirus colocalize with and disrupt PML-associated nuclear bodies at very early times in infected permissive cells. J Virol 71:4599–4613.

Arcangeletti MC, De Conto F, Ferraglia F, Pinardi F, Gatti R, Orlandini G, Calderaro A, Motta F, Medici MC, Valcavi P, Razin SV, Chezzi C, Dettori G. 2003. Human cytomegalovirus proteins pp65 and ICP72 are targeted to distinct compartmental regions in nuclei and nuclear matrices of infected human embryo fibroblasts. J Cell Biochem 90:1056–1067.

Arcangeletti MC, Pinardi F, Missorini S, De Conto F, Conti G, Portincasa P, Scherrer K, Chezzi C. 1997. Modification of cytoskeleton and prosome networks in relation to protein synthesis in influenza A virus-infected LLC-MK2 cells. Virus Res 51:19–34.

Bain M, Sinclair J. 2007. The phase of the cell cycle and its perturbation by human cytomegalovirus. Rev Med Virol 17:423–434.

Banerjee R, Weidman MK, Navarro S, Comai L, Dasgupta A. 2005. Modifications of both selectivity factor and upstream binding factor contribute to poliovirus-mediated inhibition of RNA polymerase I transcription. J Gen Virol 86:2315–2322.

Besse S, Puvion-Dutilleul F. 1996. Intranuclear retention of ribosomal RNAs in response to herpes simplex virus type 1 infection. J Cell Sci 109:119–129.

Bevington JM, Needham PG, Verrill KC, Collaco RF, Basrur V, Trempe JP. 2007. Adeno-associated virus interactions with h23/nucleoposin: Identification of sub-nucleolar virion regions. Virol 357:102–113.

Boisvert FM, van Koningsbruggen S, Navascues J, Lamond AL. 2007. The multifunctional nucleolus. Nat Rev Mol Cell Biol 8:574–585.

Boyne JR, Whitehouse A. 2006. Nucleolar trafficking is essential for nuclear export of intronless herpesvirus mRNA. Proc Natl Acad Sci USA 103:15190–15195.

Britt WJ, Auger D. 1986. Human cytomegalovirus virion-associated protein with kinase activity. J Virol 59:185–188.

Calle A, Ugrinova I, Epstein AL, Bouvet P, Diaz JJ, Greco A. 2008. Nucleolin is required for an efficient herpes simplex virus type 1 infection. J Virol 82:4762–4773.

Chen H, Wurm T, Britton P, Brooks G, Hiscox JA. 2002. Interaction of the coronavirus nucleoprotein with nucleolar antigens and the host cell. J Virol 76:5233–5250.

Coarhane AW, Chen C-H, Rosen CA. 1990. Specific interaction of the human immunodeficiency virus rev protein with a structured region in the ENV mRNA. Proc Natl Acad Sci USA 87:1198–1202.

Dal Monte P, Bessia C, Landini MP, Michelson S. 1996. Expression of human cytomegalovirus ppUL83 (pp65) in a stable cell line and its association with metaphase chromosomes. J Gen Virol 77:2591–2596.

Diaz JJ, Giraud S, Greco A. 2002. Alteration of ribosomal protein maps in herpes simplex virus type 1 infection. J Chromatogr B 771:237–249.

Dickinson LA, Kohwi-Shigematsu T. 1995. Nucleolin is a matrix attachment region DNA-binding protein that specifically recognizes a region with high base-unpairing potential. Mol Biol Cell 15:456–465.

Dove BK, You JH, Reed ML, Emnett SR, Brooks G, Hiscox JA. 2006. Changes in nucleolar morphology and proteins during infection with the coronavirus infectious bronchitis virus. Cell Microbiol 8:1147–1157.

Gallina A, Simoncini L, Garbelli S, Percivalle E, Pedrali-Noy G, Lee KS, Erikson RL, Plachter B, Gerna G, Milanesi G. 1999. Polo-like kinase 1 as a target for human cytomegalovirus pp65 lower matrix protein. J Virol 73:1468–1478.

Gorski SA, Dundr M, Misteli T. 2006. The road much traveled: Trafficking in the cell nucleus. Curr Opin Cell Biol 18:284–290.

Grinstein E, Shan Y, Karawajew L, Snijders PJ, Meijer CJ, Royer HD, Wernet P. 2006. Cell cycle-controlled interaction of nucleolin with the retinoblastoma protein and cancerous cell transformation. J Biol Chem 281:22223–22235.

Hiscox JA. 2002. The nucleolus—a gateway to viral infection? Arch Virol 147:1077–1089.

Ishov AM, Stenberg RM, Maul GG. 1997. Human cytomegalovirus immediate early interaction with host nuclear structures: Definition of an immediate transcript environment. J Cell Biol 138:5–16.

Jarkso O, Medzhidova AA, Fedorova NE, Kusch AA, Zatsepina OV. 2002. Immunocytochemical reorganization of the nucleolus in human embryo fibroblasts infected with cytomegalovirus in vitro. Dokl Biol Sci 387:589–592.

Jarkso O, Barsukova AS, Medzhidova AA, Fedorova NE, Kusch AA, Zatsepina OV. 2003. Activation of transcription of ribosome genes following human embryo fibroblast infection with cytomegalovirus in vitro. Tsitologiya 45:690–701.

Jordan P, Carino-Fonseca M. 1998. Cisplatin inhibits synthesis of ribosomal RNA in vitro. Nucleic Acids Res 26:2831–2836.

Jordan P, Mannervik M, Tora L, Carino-Fonseca M. 1996. In vivo evidence that TATA-binding protein/SL1 colocalizes with UBF and RNA polymerase I when RNA synthesis is either active or inactive. J Cell Biol 133:225–234.

Kamil JP, Coen DM. 2007. Human cytomegalovirus protein kinase UL97 forms a complex with the tegument phosphoprotein pp65. J Virol 81:10659–10668.

Lawrence FJ, McStay B, Matthews DA. 2006. Nucleolar protein upstream binding factor is sequestered into adenovirus replication centres during infection without affecting RNA polymerase I location or ablating RNA synthesis. J Cell Sci 119:2621–2631.

Malyavantham KS, Bhattacharya S, Alonso WD, Acharya R, Berezney R. 2008. Spatio-temporal dynamics of replication and transcription sites in the mammalian cell nucleus. Chromosoma 117:553–567.

Matthews DA. 2000. Adenovirus protein V induces redistribution of nucleolin and B23 from nucleolus to cytoplasm. J Virol 75:1031–1038.

Michienzi A, De Angelis FG, Bozzoni I, Rossi JG. 2006. A nucleolar localizing REV binding element inhibits HIV replication. AIDS Res Ther 3:13.

Misteli T. 2008. Physiological importance of RNA and protein mobility in the cell nucleus. Histochem Cell Biol 129:5–11.

Mittnacht S, Weinberg RA. 1991. G1/S phosphorylation of the retinoblastoma protein is associated with an altered affinity for the nuclear compartment. Cell 65:381–393.

Miyazaki Y, Takamatsu T, Nosaka T, Fujita S, Martin TE, Hatanaka M. 1995. Function of human immunodeficiency virus type 1 Rev: Implications for its interaction with the nucleolar protein B23. Exp Cell Res 219:93–101.

Okuwaki M, Matsumoto K, Tsujimoto M, Nagata K. 2001. Function of the cytotoxicity of human immunodefsive virus type 1 Rev: Implications between DNA replication and transcription. J Cell Biochem 94:554–565.

Pokrovskaja K, Mattsson K, Kashuba E, Klein G, Szekely L. 2001. Proteasome inhibitor induces nucleolar translocation of Epstein-Barr virus-encoded EBNA-5. J Gen Virol 82:345–358.

PONTI D, Troiano M, Bellenchici GC, Battaglia PA, Gigliani F. 2008. The HIV Tat protein affects processing of ribosomal RNA precursor. BMC Cell Biol 9:32–41.

Ruggero D, Pandolfi PP. 2003. Does the ribosome translate cancer? Nat Rev Cancer 3:179–192.
Russell J, Zomerdijk. 2005. RNA-I-directed rDNA transcription, life and works. Trends Biochem Sci 30:90–94.
Sanchez V, Angeletti PC, Engler JA, Britt WJ. 1998. Localization of human cytomegalovirus structural proteins to the nuclear matrix of infected human fibroblasts. J Virol 72:3321–3329.
Shimakami T, Honda M, Kusakawa T, Murata T, Shimotohn K, Kaneko S, Murakami S. 2006. Effect of hepatitis C virus (HCV) NS5B-nucleolin interaction on HCV replication with HCV subgenomic replicon. J Virol 80:3332–3340.
Simonin D, Diaz JJ, Massè T, Madjar JJ. 1997. Persistence of ribosomal protein synthesis after infection of HeLa cells by herpes simplex virus type 1. J Gen Virol 78:435–443.
Siomi H, Shida H, Maki M, Hatanaka M. 1990. Effects of a highly basic region of human immunodeficiency virus Tat protein on nucleolar localization. J Virol 64:1803–1807.

Somogyi T, Michelson S, Masse MG. 1990. Genomic location of a human cytomegalovirus protein with protein kinase activity. Virol 174:276–285.
Tanaka S, Fukurawa T, Plotkin S. 1975. Human cytomegalovirus stimulates host cell RNA synthesis. J Virol 15:297–304.
Ugrinova I, Monier K, Ivaldi C, Thié M, Storck S, Mongelard F, Bouvet P. 2007. Inactivation of nucleolin leads to nucleolar disruption, cell cycle arrest and defects in centrosome duplication. BMC Mol Biol 8:66–82.
Verdun DH. 2006. The nucleolus: A model for the organization of nuclear functions. Histochem Cell Biol 126:135–148.
Yun JP, Chew EC, Liew CT, Chan JY, Jin ML, Ding MX, Fai YH, Li HK, Liang XM, Wu QL. 2003. Nucleophosmin/B23 is a proliferate shuttle protein associated with nuclear matrix. J Cell Biochem 90:1140–1148.