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Review

Viruses and the nucleolus: The fatal attraction[☆]Anna Salvetti^{*}, Anna Greco^{*}

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ABSTRACT

Viruses are small obligatory parasites and as a consequence, they have developed sophisticated strategies to exploit the host cell's functions to create an environment that favors their own replication. A common feature of most – if not all – families of human and non-human viruses concerns their interaction with the nucleolus. The nucleolus is a multifunctional nuclear domain, which, in addition to its well-known role in ribosome biogenesis, plays several crucial other functions. Viral infection induces important nucleolar alterations. Indeed, during viral infection numerous viral components localize in nucleoli, while various host nucleolar proteins are redistributed in other cell compartments or are modified, and non-nucleolar cellular proteins reach the nucleolus. This review highlights the interactions reported between the nucleolus and some human or animal viral families able to establish a latent or productive infection, selected on the basis of their known interactions with the nucleolus and the nucleolar activities, and their links with virus replication and/or pathogenesis. This article is part of a Special Issue entitled: Role of the Nucleolus in Human Disease.

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1. Introduction

Viruses are small obligatory parasites and as a consequence, they have to divert some of the cellular machineries for their own replication. They have developed sophisticated strategies to exploit the host cell's functions and to inhibit its intrinsic and innate defense mechanisms in order to efficiently accomplish their replication cycle. Viral infections are generally associated with specific diseases affecting one or several organs or tissues, some of which can be fatal for the host. Accordingly, studying the interaction between viruses and the cell is extremely informative, not only to understand the virus properties but also to gain a better insight into the cell's functions.

The viral genome is a DNA or RNA molecule that encodes viral components that allow a latent/chronic or lytic infection. Generally, most DNA viruses replicate in the nucleus while most RNA viruses replicate in the cytoplasm. However, exceptions also exist with some DNA viruses and RNA viruses replicating in the cytoplasm and the nucleus, respectively. During latent or chronic infection, only a few viral components are synthesized and the viral genome persists in the infected cell. A typical infectious cycle is usually lytic. It includes attachment of the virus to the cell surface using specific receptor, entry through the plasma membrane to reach the cytoplasm, production of viral RNAs and proteins, genome replication, and at the end of the cycle the newly made viral components

are assembled into progeny virus particles that are released from the infected cells and spread to new cells.

The consequences of viral infection on host cell functions are diverse. Surprisingly, despite the important variety of mechanisms, a common feature of most – if not all – viral families is their interaction with the nucleolus, one of the best-known nuclear compartments [1–4]. The interaction of viruses with the nucleolus has been the object of an increasing number of studies since the beginning of the 1990s, some of them establishing a link between their ability to interact with this nuclear compartment and the outcome of virus replication and pathogenesis.

The nucleolus, the most prominent nuclear domain, is a membrane-less structure whose existence was established in the 19th century. Until recently, its most well known role was ribosome biogenesis. Indeed, the nucleolus forms around the clusters of genes coding for ribosomal RNAs arranged in a tandem array, and the transcriptional activity of ribosomal genes in the nucleolus gives rise to its characteristic ultra-structural organization: the fibrillar center, surrounded by the dense fibrillar component, which is bordered by the granular component [5]. During mitosis the nucleolus disassembles, then reassembles at the end of mitosis. Subsequently, the nucleolus was discovered to be more than a “ribosome factory” [6]. Studies in the last decades have identified several thousands of different nucleolar components (proteins and RNAs) the roles of which have highlighted that the nucleolus is also involved in other biological functions such as tRNA and mRNA processing, maturation and assembly of ribonucleoprotein complexes, cell cycle regulation and cellular aging, leading to the notion of a plurifunctional nucleolus. In addition, nucleoli are dynamic nuclear domains and their components communicate constantly with other nuclear domains and with the cytoplasm [4,7–12].

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Therefore, due to the multiple functions fulfilled by nucleoli, it is not surprising that in cells infected with various types of viruses, nucleoli are submitted to profound alterations in structure and composition. Indeed, in addition to the numerous viral components that traffic to and from the nucleolus, some nucleolar proteins are delocalized out of the nucleolus, while in other cases non-nucleolar cellular proteins enter the nucleolus to fulfill other function(s) [1,3]. At present, the roles of these virally-induced nucleolar perturbations on viral replication and host cell functions are not fully elucidated for many of them. Even though the infected cells need to support the synthesis of new viral proteins, only a few studies on viral infection focus on mechanisms related with ribosome biogenesis demonstrating that viral proteins interact with rRNAs, inhibit or stimulate rRNA gene transcription, or modulate pre-rRNA maturation [13–17]. By contrast, numerous studies have shown that several of the virally-induced modifications of nucleolar structure and composition rather interfere with other well established fundamental processes in which they are directly or indirectly involved, such as cell cycle regulation, apoptosis and translation. In addition, these studies showed that nucleoli themselves or nucleolar proteins participate directly in specific processes that are crucial for the outcome of infection, like viral DNA replication, virus assembly, and control of intracellular trafficking.

The aim of this review is to highlight the interactions reported between the nucleolus and some viral families, which illustrate the variety of studies in this field and of their potential relevance to the development of treatments against viral infections. To simplify this potentially huge task, we made the choice to focus on a discrete number of viral families chosen for their importance in human or animal disease and their mode of replication. In particular, this review will focus on some single-stranded RNA viruses belonging to the Flaviviridae, Coronaviridae and Togaviridae families, and double-stranded DNA viruses belonging to the Herpesviridae family, which represent viruses replicating in the cytoplasm or the nucleus of the infected cell, respectively. An abundant literature, including several reviews has already been published on the interaction between retro- and lenti-viruses, such the Human Immunodeficiency Virus (HIV), with the nucleolus [18–23]. There is also increasing data showing that plant viruses hijack the nucleolus to promote virus replication [24,25]. The information available on these latter viruses was deliberately omitted and we invite the readers to refer to specific articles for more detailed information on this topic.

2. The nucleolus: a central hub for the replication of pathogenic RNA viruses?

The majority of RNA viruses replicate in the cytoplasm of the infected cell where all the infectious cycle takes places, including transcription, replication of the RNA genome and assembly of newly infectious particles. Not surprisingly however, several studies have additionally described the interaction of a number of these viruses with the nucleus and in particular the nucleolus [26]. This chapter will focus on four different families of RNA viruses that possess a positive (+) strand RNA genome (Table 1). These four families contain viruses that are highly pathogenic in animals and/or primates, including man, and, consequently, most of them have been the focus of recent intensive studies. This is the case, in particular, for members of the Flaviviridae family such as Hepatitis C virus (HCV), a widely spread human virus which causes a chronic infection of the liver which can lead to cirrhosis and hepatocellular carcinoma [27], or the arthropod-transmitted viruses, Dengue virus (DENV), West Nile Encephalitis virus (WNV) or Japanese Encephalitis virus (JEV), which can cause severe hemorrhagic or neurological syndromes in man [28]. Members of the Coronaviridae and Arteriviridae families such as the Severe Acute Respiratory Syndrome coronavirus (SARS-CoV), the avian Infectious Bronchitis virus (IBV) and the Porcine Reproductive and Respiratory Syndrome virus (PPRSV) are also considered major pathogens causing severe respiratory diseases in man and animals [29]. Finally, it is of particular interest to

also cite the interactions reported for two members of the Togaviridae family, the Semliki Forest Virus (SFV) and the Getah-like alphavirus (GETV) M1 which, even if not considered major pathogens for man, have attracted interest as anti-cancer tools [30] and could also, by extension, predict future interesting interactions for other more pathogenic members of this viral family such as the Chikungunya virus.

2.1. Replicative cycle of positive-strand RNA viruses

Positive-strand RNA viruses are composed of a lipid envelope containing the viral glycoproteins responsible for attachment to the cell membrane and penetration, surrounding a capsid that contains the RNA genome. The size and the shape of the assembled capsid can vary according to the virus but a common feature is that it is composed of multiple copies of a unique protein, called capsid, nucleocapsid (N), or core, which is able to bind and condense RNA and thus constitutes a protective shell for the viral genome. After attachment to the cell surface and delivery of the RNA in the cytoplasm, the viral genome is immediately translated into the enzymes required for its replication, which occurs *via* a negative (–) strand RNA intermediate. Newly replicated viral RNA molecules are used for the synthesis of the viral proteins and as a substrate during particle assembly. All these processes are accomplished by exploiting virus-encoded enzymes and cellular components, in particular cellular membranes which are involved in the formation of particles from intra-cytoplasmic organelles, mainly the endoplasmic reticulum and Golgi apparatus [27].

2.2. Viral factors interacting with the nucleolus

Despite the diversity of proteins encoded by the genomes of (+) strand RNA viruses, it is striking to observe that most of the reported interactions with the nucleolus concern the same structural protein, namely the capsid, which under different names has several common properties among all viral families including its small size (generally <50 kDa), clusters of basic amino acids (aa), and its ability to bind viral and sometimes cellular RNA. It is unclear if this finding reflects a true predilection of the nucleolus for this structural component or if it simply results from the fact that this is one of the most abundant viral proteins which is, therefore, easier to detect in particular in a compartment such as the nucleolus where the proteins rapidly shuttle in and out. Interestingly, some studies have reported the presence of non-structural viral proteins in the nucleolus. This is the case for the accessory protein 3b from the SARS-CoV, which was found to predominantly localize in the nucleolus [31,32]. Further studies indicated that this protein, which inhibits type I interferon (IFN I) production, could shuttle from the nucleus and mitochondria but, surprisingly, there was no further investigation as to its nucleolar localization [33]. Another example is provided by the nsP2 protein of SFV, which is a multifunctional protein essential for viral replication and maturation which was found localized mostly in the nucleus and nucleoli [34,35]. Surprisingly, again, this latter property was not re-investigated in further studies, which focused exclusively on its nuclear localization [33]. Lastly, deletion of the membrane-anchoring domain of the RNA-dependent RNA polymerase (RdRP) NS5B of HCV induced the delocalization of the protein in the nucleolus also suggesting that this viral enzyme contained a cryptic nucleolar localization signal (NoLS), allowing its transient traffic through the nucleolus [36].

2.3. Mechanisms of nucleolar import and export of viral proteins

Localization of viral proteins in the nucleolus is frequently not exclusive and sometimes hard to visualize. In some cases, nucleolar localization was revealed or enhanced by introducing deletions into domains of the protein suggesting that the signals involved in nucleolar targeting were masked by other domains or that this subcellular localization is restricted to some cleaved forms. This was particularly evident for the

Table 1
Interactions of selected (+) strand RNA viruses with the nucleolus.

| Virus family | Virus Name | Viral factors ^a | Cellular proteins ^b | Effects on the host and/or the virus | References |
|---------------|----------------------------|---------------------------------|--------------------------------|---|---|
| Flaviviridae | DENV | Capsid | HDM2, DDX56, Jab1 | DDX56 important for virus infectivity HDM2 localized in the nucleolus and induction of p53-dpt apoptosis Jab1 protects cells against the capsid cytotoxic effects | [41,42] |
| | WNV | Capsid (123 aa) | | | [43,44,60,61] |
| | JEV Kunjin virus HCV | Capsid Capsid Core, NS5B? | B23 Nucleolin, B23, PKR | B23 important for virus replication Upregulation of B23 synthesis <i>via</i> reduction of YY1 repressive activity on B23 promoter NCL is important for virus replication NCL found at HCV IRES | [45,130] [46] [36–38,53,62,63,70,131] |
| Coronaviridae | SARS-CoV | 3b, Nucleocapsid | B23 | Inhibition of B23 phosphorylation Alteration of fibrillarin localization p53 delocalized in the perinuclear region Inhibition of cell growth | [31,32,39,40,132] |
| | Avian IBV | Nucleocapsid | Nucleolin, p53 | | [47,48,133,134] |
| Arteriviridae | PPRSV | Nucleocapsid | Fibrillarin, HIC | Mutation of N prevents its nucleolar localization reduces viral replication <i>in vitro</i> , delays viremia and increases NAb titers <i>in vivo</i> . | [17,49,51,52,135] |
| Togaviridae | SFV | nsP2, capsid | | Mutation of nsP2 prevents its nuclear localization and reduces viral spread and neurovirulence <i>in vivo</i> | [34,35,57,73–76,136] |
| | GETV M1 | | P21waf | S-phase arrest and apoptosis in glioma cells | [72] |

^a Viral proteins shown to localize in the nucleolus.

^b Nucleolar proteins interacting with viral factor or cellular, non nucleolar proteins shown to be translocated into the nucleolus by the virus or the viral proteins.

capsid proteins, which for many viruses naturally exist in immature and mature forms produced by cleavage of N- or C-terminal domains. For example, the Core protein from HCV which normally mainly localizes in the cytoplasm where it associates with the endoplasmic reticulum and lipid droplets, was found predominantly in the nucleus and the nucleolus upon deletion of the C-terminal hydrophobic region, thus confirming previous data showing the presence of this protein in the nucleus and the nucleolus of hepatocytes from chronically infected HCV patients [37,38]. Similarly the SARS-CoV N protein mainly localized in the cytoplasm of the infected cells but could be visualized in the nucleus, and particularly in the nucleolus, using deletion mutants [39,40]. By contrast, the DENV capsid protein could be clearly observed in the nucleoli of cells expressing this protein alone or infected with the virus [41,42]. Similar observations were made for the capsid protein from other arthropod-borne viruses such as those derived from WNV [43,44], JEV [45], and Kunjin virus [46] as well as from several members of the Coronaviridae and Arteriviridae families [47–49].

Many parameters control the nucleolar localization of these multi-functional proteins. In particular their presence in the nucleolus varied according to the mode of nuclear import, the interaction with nucleolar components and the kinetics of nuclear import and export. Despite the fact that most of the capsid proteins are small in size, and thus potentially able to passively diffuse through the nuclear pores, an active energy-dependent mechanism was demonstrated to be responsible for their nuclear entry. Accordingly, nuclear localization signals (NLS) were identified in nearly all the capsid proteins. Identification of a NoLS proved more problematic since a consensus NoLS is not available and in several examples the signal required to target the protein to the nucleolus was imbedded in the NLS-containing regions. However, in some cases nucleolar import of capsid proteins was shown to be mediated by distinct and well-identified NoLS as is the case for the JEV, IBV, and PPRSV capsid proteins [45,50–52]. Besides the presence of basic amino acid residues, identified NoLS sequences are all different and most of them likely act by mediating the interaction with cellular proteins and/or RNAs that transit through the nucleolus or are constitutive components of this nuclear body such as nucleolin or B23 [53].

Similarly to their import in the nucleus and the nucleolus, other domains of these proteins are responsible for their export into the cytoplasm, the site where the assembly of infectious particles takes place. Not surprisingly, several groups identified nuclear export signals (NES), which mediate the translocation of these proteins into the cytoplasm *via* a CRM1-dependent [54] or -independent mechanism [55,56]. Alternatively, nucleolar/nuclear export can be due to association with

cellular factors as is the case for the capsid protein of WNV, which is translocated to the cytoplasm when associated with Jab 1 [43]. Interestingly, the rate of nuclear/nucleolar import *versus* export correlated with the predominant localization of the protein. In particular, some studies have revealed that import of the capsid proteins of SFV and DENV into the nucleolus was very rapid and that it could occur at very early stages during infection before the assembly of infectious particles [41,57]. Live cell imaging associated with photo-bleaching experiments further indicated that Arterivirus capsid protein was not permanently sequestered into the nucleolus and that the apparently higher distribution of this protein in the nucleolus relative to the cytoplasm, when expressed alone, was due to a higher nuclear import rate [58]. Interestingly, import rates into the nucleolus varied according to the NoLS sequence considered [59].

2.4. Role of interactions of viral factors with the nucleolus or the nucleolar components

The reason why proteins from cytoplasmic RNA viruses localize to the nucleolus is presently unclear and several nonexclusive hypotheses can be proposed. First, this phenomenon could be seen as an innate cellular defense aiming to retrieve viral proteins away from the cytoplasm where virus replication and assembly occur. However, this hypothesis does not fit with the observation that viral proteins usually are not permanently sequestered in the nucleolus and that their passage through this nuclear compartment is a very dynamic process. In addition, the amount of viral proteins found in the nucleolus can be extremely low and, thus, not compatible with an efficient anti-viral mechanism. Alternatively, passage through the nucleolus could be a way for the cell to post-translationally modify the viral proteins and to inhibit or modify their function. Indeed, capsid proteins from most RNA viruses are not only structural factors involved in virion assembly but also multi-functional regulatory proteins involved in critical processes such as the control of cell division and apoptosis. Still, this hypothesis is not consistent with the observation that in many situations the interaction of viral proteins with the nucleolus was demonstrated to be important for efficient viral replication.

Therefore, the nucleolus is rather considered as playing a positive role in viral replication. In particular, the interaction with the nucleolus could be a way for the virus to export into the cytoplasm nucleolar factors required for its replication. Many examples exist among viruses of the Flaviviridae family. The capsid protein of WNV was shown to bind to the nucleolar RNA helicase DDX56 and relocate it to the cytoplasm

where this cellular factor played a role in a post-replicative step of virus assembly [60,61]. Accordingly, knockdown of DDX56 using siRNA induced a more than 100 fold decrease in the production of infectious particles and over-expression of the capsid-binding region of DDX56 severely reducing the infectivity of the virus thus opening interesting perspectives for future therapeutic interventions. Similarly, the core protein of JEV was reported to interact with and delocalize the nucleolar protein B23 to the cytoplasm. Nucleolin, another abundant nucleolar protein, was found to co-localize with the NS5B protein of HCV in the perinuclear region and a truncated form of NS5B, lacking the membrane-anchoring domain, co-localized with nucleolin in the nucleolus. This suggests that the wild type viral protein was able to transit through the nucleolus and delocalize nucleolin in the cytoplasm. Accordingly, knockdown of nucleolin reduced HCV replication [36,62,63].

Besides providing cellular factors for viral replication, interaction with the nucleolus may indirectly help the virus by modifying the cell's status. Several interesting studies point to a relationship between the interaction of viruses with the nucleolus and apoptosis. WNV is known to trigger cell death through either apoptosis or necrosis [64,65]. However, as compared to other viruses, WNV has a relatively long replicative cycle and apoptosis occurs only at late stages of the infectious process [66]. Older studies indicated that the capsid protein could induce p53-dependent apoptosis by sequestering HDM2 into the nucleolus [44]. This effect, however, may be counterbalanced by the interaction of capsid with Jab1, a subunit of the COP9 signalosome complex, which can delocalize the WNV capsid in the cytoplasm, induce its degradation and prevent its cytotoxic effect [43]. Importantly, a recent study indicated that a shorter (105 aa) isoform of the WNV capsid, without the 18-aa signal peptide corresponding to the mature protein found in infected cells, rather exerted an anti-apoptotic effect [66]. Interestingly, the first 15 aa at the N-terminus of the immature capsid were found to mediate interaction with Jab1 [67]. Altogether, these results suggest that several mechanisms exist to control the pro-apoptotic effect of the longer (123 aa) immature capsid protein, which was described to go the nucleolus. The mature form (105 aa) blocks apoptosis, probably to allow sufficient time for the virus to replicate. It is likely that the mature capsid is also able to localize in the nucleolus and further studies should be performed to determine the effect of the nucleolar localization of the capsid on this phenomenon. Similar debate on the pro- or anti-apoptotic activities exists for the core protein of HCV [68]. Induction or not of apoptosis is of crucial importance to understand the mechanisms underlying both the liver damage induced by the virus during chronic infection with HCV and carcinogenesis. The core protein of HCV is considered to be a potential oncoprotein [69]. As for the WNV capsid, several isoforms of the HCV core exist, which derive from an immature full length protein that is sequentially cleaved into truncated proteins, the latter being able to localize to the nucleus and the nucleolus [70]. Studies conducted on the core protein have shown that it could both induce and counteract apoptosis [38,71]. In particular, with regard to its interaction with the nucleolus, Realdon et al. have shown that the expression of the truncated version of the core protein alone induced higher levels of apoptosis than the full-length protein. In addition, induction of apoptosis could be related to translocation of PKR into the nucleolus [38]. A later interesting example of apoptosis induced upon translocation of a cellular protein in the nucleolus derives from the study of the Getah-like alphavirus M1. Infection of glioma cells with the M1 alphavirus was shown to induce arrest of the cells in S phase and apoptosis. This effect was further shown to be due to a down-regulation of the cyclin-dependent kinase inhibitor p21Waf1, possibly through its translocation into the nucleolus [72]. Interestingly, studies conducted on another member of the Alphaviridae family, the SFV, have shown that both the non-structural protein nsP2 and the capsid can localize to the nucleolus [34,35,57,73,74] and that abrogation of the capacity of nsP2 to localize in the nucleus reduced the cytotoxic effect of the virus [75]. Therefore, it is likely that even for the M1 alphavirus, translocation of p21Waf1 into the

nucleolus may be due to its direct or indirect association with a viral constituent.

2.5. Effect on virus induced pathogenesis

JEV is the leading cause of arthropod-borne virus encephalitis in Asia. As with nearly all Flaviviruses, the mature capsid protein is localized not only in the cytoplasm but also in the nucleolus. A very interesting study examined the effect of point mutations in the capsid protein that affected its ability to localize in the nucleolus. Viruses bearing such mutations produced a core protein, which was exclusively cytoplasmic in both insect and mammalian cells. Interestingly, the analysis of this mutant virus *in vitro*, indicated that it was impaired for replication, in particular in mammalian cells with more than 100 fold lower titers than those reached with the wild type virus and a larger number of defective particles [45]. In addition, revertant viruses rapidly emerged *in vitro*, indicating that nucleolar localization was important for virus growth. Reduced viral growth and the appearance of revertants were also observed after direct intra-cerebral inoculation of the mutant virus in mice. Neurovirulence, however, was not affected and even increased with the mutant virus. By contrast, neuroinvasiveness, measured by its ability to reach the central nervous system (CNS) after peripheral inoculation, was severely affected [45]. This criterion reflects the ability of the virus to replicate in the peripheral organs, in particular in the lymphatic tissues, before crossing the blood–brain barrier. Therefore, it is possible that the default in the nucleolar localization of the JEV capsid protein prevented replication of the virus in the peripheral tissues at a level sufficient to access the CNS. Similarly a reduced mortality was observed after intra-cerebral injection of a SFV strain coding for a mutated nsP2 protein impaired for its nuclear localization [76].

Another very interesting example is provided by the study of the PPRSV N protein. PPRSV is the causative agent of a severe infectious disease of swine that causes significant economic losses in the pig industry. Lee et al. examined the effect of a mutation affecting the nuclear and nucleolar localization of N protein in infected cells. Mutant viruses displayed a reduced replication resulting in a 100-fold decrease in viral titers. More importantly, intranasal inoculation of virus in pigs indicated that the mutant form delayed viremia and induced a higher level of neutralizing antibodies. Interestingly, a mutation at the NLS locus of N, enabling the protein to go to the nucleolus, was detected in the virus extracted from the tonsils of all the animals injected with the mutant virus. This latter result indicated that a strong selection pressure had been applied to this region of N, in order to allow persistence of the virus *in vivo*. Interestingly, further studies with a reversion-resistant mutant virus confirmed the previous observation and further indicated that mutant virus persisted in the tonsils at a reduced level [77,78].

3. Nucleolar modifications induced by herpes viruses: cellular proteins that leave or reach the modified nucleolus participate in virus life and/or alteration of cellular processes

This part of the manuscript is dedicated to studies in the field of infection by herpes viruses, especially herpes simplex virus type 1 (HSV-1), human cytomegalovirus (HCMV), and Kaposi sarcoma-associated herpes virus (KSHV) also known as human herpesvirus 8 (HHV8). Several well-documented data have already illustrated the important role played by ORF57 encoded by KSHV during the lytic infection and the function of its nucleolar localization in the nuclear export of intronless viral RNAs [79–81]. In this chapter we will instead focus on the role of nucleolin, the most abundant nucleolar protein that leaves the nucleolus during HSV-1 and HCMV infection to reach the viral replication compartments (VRCs) and of angiogenin, a secreted non-nucleolar protein which is up-regulated after KSHV infection and then targeted to the nucleolus.

Herpes viruses have a large DNA genome and replicate in the cell nucleus. After primary infection, herpes viruses have the ability to remain in a latent state *in vivo*, which is characterized by the persistence of the

viral genome, the expression of a limited number of genes and the absence of virus production. The latent virus, which persists for the life span of the host, can be reactivated periodically, and the viral immediate-early, early, and late genes expressed in a coordinated fashion giving rise to a lytic productive viral cycle, which leads to the production of infectious particles and eventually to cell death due to lysis [82]. Viral proteins expressed during the latent and the lytic phases contribute to the pathogenesis of the virus-associated diseases. Many herpes virus infections are responsible for cutaneous manifestations [83]. Among herpes viruses, HCMV is an important pathogen, and HCMV infection is considered as the most common cause of human congenital microbial infections. Recent reports also suggest that HCMV is associated with some human malignancies [84]. Immunocompromised patients develop severe HSV and HCMV infections with significant morbidity and mortality [85,86]. KSHV, which was discovered in 1994, is the causative agent of Kaposi's sarcoma that occurs frequently in immunosuppressed patients. The lesions of Kaposi sarcoma are characterized by a proliferation of small vessels surrounding more ectatic vessels induced by angiogenic factors.

3.1. Nucleolin is delocalized in viral replication compartments in HSV-1- and HCMV-infected cells and fulfills different functions

Nucleolin is the most abundant and probably most-studied protein of the nucleolus and has been shown to shuttle from the nucleolus to the nucleoplasm, the cytoplasm, and the plasma membrane. It is a multifunctional protein that undergoes many post-translational modifications, including phosphorylation, glycosylation, and acetylation that relate to its localization and function(s). In addition to its role in ribosome biogenesis in the nucleolus, it participates in many essential cellular processes, such as chromatin remodeling, DNA recombination and replication, RNA transcription by RNA Pol I and II, rRNA processing, mRNA metabolism, cell proliferation, cytokinesis, and apoptosis [87–91]. Nucleolin is involved in the infection process of numerous RNA and DNA viruses where it plays important roles during different steps of the viral life cycle. It binds directly or indirectly to viral factors and is involved in the viral life cycle and, therefore, in virus-associated pathogenesis [62,63,92–95]. For example, nucleolin interacts *in vitro* with the NS1 protein of influenza A virus, and it co-localizes with NS1 protein in infected cells. However, its role is not yet known [92]. Nucleolin present at the surface of some types of cells is a co-receptor for the entry of HIV, human parainfluenza virus type 3, respiratory syncytial virus, and probably of Crimean-Congo hemorrhagic fever virus and of Japanese encephalitis virus [96–100]. Knockdown of nucleolin mobilizes adeno-associated virus particles to the nucleoplasm [101]. Nucleolin interacts with several viral RNAs and is suspected of regulating viral and cellular RNA metabolism, including splicing and translation [102–104]. Nucleolin also has the ability to interact with viral genomic RNAs and to positively or negatively regulate viral replication [63,105,106]. Nucleolin is also linked to cervical carcinoma induced by human papilloma virus 18 by controlling the expression of viral oncogenes in a cell cycle-dependent manner [107,108].

In HSV-1 and HCMV-infected cells, the formation of the VRCs in the nucleus of infected cells is accompanied by a profound modification of the structure and the composition of nuclear domains, including the nucleolus. Many nucleolar proteins are delocalized out of the nucleolus. This is the case for nucleolin that is targeted to the VRCs of these two viruses, and it participates in different aspects of their life cycle.

3.1.1. Nucleolin is involved in HSV-1 nuclear egress

Soon after HSV-1 infection, nucleoli undergo drastic morphological and structural changes. Nucleolin, B23/NPM, fibrillarin, UBF, and RPA194 nucleolar proteins progressively leave the nucleolus; nucleolin, B23, and UBF are delocalized into the VRCs, which are the sites of replication, transcription, and encapsidation of HSV-1 genomes [109–111]. During HSV-1 infection nucleolin expression is up regulated contrary

to most of the cellular proteins that are down regulated. This suggests that nucleolin is required for the outcome of infection. The delocalization of nucleolin out of the nucleolus is under the control of the UL24 viral protein [110]. Moreover, viral infection and viral production are inhibited in cells where nucleolin is knocked down, indicating that nucleolin is required for HSV-1 life cycle [109]. A series of convergent results from independent laboratories strongly suggests that nucleolin is involved in the nuclear egress of viral particles at the end of the viral cycle by a mechanism that is not yet elucidated. Indeed, inhibition of nucleolin expression reduced capsid accumulation as well as the amount of encapsidated viral DNA in the cytoplasm of infected cells [112]. In addition, nucleolin was present in a protein complex containing UL12 viral protein that was suspected of being involved in viral DNA maturation and nuclear egress [112]. Further studies indicated that nucleolin interacted directly with the structural US11 viral protein and was required for nucleocytoplasmic shuttling of US11 [113]. Therefore, the association of nucleolin with these two viral proteins upholds its role in HSV-1 egress.

3.1.2. Nucleolin is required for maintaining the architecture of HCMV replication compartments

Nucleolin is also important for the life cycle of HCMV where it contributes to the organization of the VRCs. As in the case of HSV-1, nucleolin is up regulated and redistributed throughout the nucleus during HCMV infection [114]. Nucleolin was found specifically associated with the viral UL44 DNA polymerase processivity factor in infected cells. UL44 could associate with nucleolin in the absence of DNA and of any other viral protein [114,115]. Nevertheless, the inhibition of nucleolin expression impaired viral DNA synthesis and virus production. UL44 is located at the periphery of the viral replication compartments where it is concentrated in a peripheral layer where viral DNA synthesis occurs. It has been shown that nucleolin surrounds UL44 at the border of the VRCs and partially co-localized with UL44 [115]. Results obtained from nucleolin knock-down cells suggest that nucleolin is required for the correct formation of the VRCs by targeting UL44 at the periphery of the VRCs which consequently promotes viral genome synthesis.

These two examples demonstrate that nucleolin plays different roles in the infection lytic process of two different viruses belonging to the same family. In both cases, knock down of nucleolin impairs viral infection and very probably the associated diseases.

3.2. Angiogenin is targeted in the nucleolus upon KSHV infection and is involved in both the replication of the virus, and in the modulation of cellular processes

The cells infected with KSHV in Kaposi sarcoma are spindle cells that display endothelial markers. KSHV is also linked to two B-cell lymphoproliferative diseases, primary effusion lymphoma and multicentric Castleman disease. KSHV latent genes drive cell proliferation, and counteract apoptosis, while both the latent and lytic viral genes induce neoangiogenic inflammatory networks. Both latent and lytic infections of KSHV play a role in tumorigenesis. It has been recently shown that KSHV infection of endothelial cells induces a high expression of angiogenin and its localization to the nucleolus [16]. This is correlated with the induction of cell proliferation and the formation of new blood vessels.

Angiogenin is a protein of 14 kDa known as a potent inducer of neovascularization as it mediates the formation of new blood vessels. Its expression is often up regulated in various cancers, and this is linked with cancer progression and poor prognosis. Angiogenin is a secreted protein, which belongs to the RNase family. However, it is endocytosed by relevant cell types, then translocated to the nucleus where it accumulates in the nucleolus. Angiogenin contains a nucleolar targeting signal corresponding to residues 31–35 [116]. Once in the nucleolus, angiogenin binds to the CT rich specific angiogenin-binding elements

identified in the gene encoding rRNA (rDNA) and stimulates rRNA synthesis and, therefore, ribosome biogenesis and cellular proliferation [117]. Internalization and translocation of angiogenin to the nucleolus are required for the induction of rDNA transcription and for its activity in angiogenesis [118–120].

Results obtained from sub-confluent endothelial cells infected *de novo* with KSHV revealed that the virally-induced angiogenin was targeted to the nucleolus where it bound to the promoter present in the 45S rDNA, increasing the synthesis of rRNA. This leads to the augmentation of the survival of KSHV-infected endothelial cells due to the anti apoptotic and proliferative effect of angiogenin [16]. Interestingly, the nucleolar localization of angiogenin was crucial for these effects, since they were greatly reduced or abolished when the nuclear translocation of angiogenin was specifically blocked [16]. Importantly, in KSHV-latently-infected cells, the inhibition of the nuclear translocation of angiogenin resulted in the inhibition of viral latent LANA-1 gene expression, in the reactivation of the latent viral genome and the induction of the lytic cycle, leading to cell death [121]. Moreover, the increased and sustained induction of angiogenin needs the expression of KSHV genes. Since the expression of the lytic ORF74 viral gene plays roles in angiogenin expression, it has been speculated that ORF74 could induce angiogenin expression [16]. A series of data suggests that KSHV utilizes angiogenin to maintain its latency, probably by activating the PLC- γ pathway [121–123].

Altogether, these data demonstrate that KSHV-induced angiogenin and its localization to the nucleolus are involved both in the control of the viral cycle, and in the modulation of different cellular pathways, including rRNA synthesis, cell proliferation, apoptosis, and angiogenesis. There are several lines of evidence showing a role for p53 in angiogenin function in infected cells [124,125]: LANA-1 and angiogenin were found in the same complexes containing p53. However, neither p53 nor another viral protein was required for LANA-1 and angiogenin interaction. In addition, blocking the nuclear transport of angiogenin resulted in the modification of p53 expression and in its intracellular redistribution in KSHV(+) cells. It has been suggested that LANA-1 and angiogenin sequester p53 in the nucleus in an inactive form, and that angiogenin, possibly through p53-mediated pathways, maintains the survival of KSHV infected cells and its latency.

4. Conclusion

Altogether, studies conducted on the interaction of viruses with the nucleolus have clearly indicated that this nuclear body plays a major role in their life cycle, independent of their replication site and of whether they are the causative agents of acute or chronic/latent infections. However, most studies have focused on very specific interactions which certainly under-estimate what is going on during natural infections. In particular, many studies have been performed by individually expressing viral proteins. Therefore, a more comprehensive view of the nucleolus-virus interaction should include analyses in the context of the whole virus and possibly *in vivo* to establish a link with the virus-induced pathology. Recent interesting work examining the large scale effect of virus infection on the nucleolar proteome provides excellent examples showing directions for future studies [124,126–129]. These approaches will help both in understanding the role of viral and cellular components that traffic in or out the nucleolus during viral infection, and designing novel classes of molecules that target these factors or any of their partners capable of inhibiting virus growth, cell proliferation, or tumor formation.

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