Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
The interaction of animal cytoplasmic RNA viruses with the nucleus to facilitate replication

Julian A. Hiscox *

School of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, UK

Abstract

A number of positive and negative strand RNA viruses whose primary site of replication is the cytoplasm use the nucleus and/or nuclear components in order to facilitate their replicative processes and alter host cell function. The nucleus itself is divided into a number of different sub-domains including structures such as the nucleolus. Many of the nuclear proteins that localise to these domains are involved in RNA processing, and because of their limited coding capacity, it may be necessary for RNA viruses to sequester such cellular factors in order to facilitate the replication, transcription and translation of their genomes. Amongst the best-studied examples of this are the picornaviruses, whose infection results in the redistribution of nuclear proteins to the cytoplasm and their interaction with the internal ribosome entry site (IRES) to facilitate translation of the picornavirus polyprotein. Examples can be found of other positive and also negative strand RNA virus proteins that localise to the nucleus and sub-domains (especially the nucleolus) during virus infection, and several localisation motifs have been defined. Apart from sequestering nuclear proteins for a role in replication, such viruses may also target the nucleus to disrupt nuclear functions and to inhibit antiviral responses.

#2003 Elsevier B.V. All rights reserved.

Keywords: RNA; Viruses; Nucleus; Nucleolus; Replication

1. Introduction

The nucleus has traditionally been viewed as the domain of retroviruses, many DNA viruses such as herpesviruses and adenoviruses, and of some negative strand RNA viruses, most notably the orthomyxoviruses and some mononegavirales such as Borna disease virus and some insect rhabdoviruses (Whittaker et al., 2000). Positive strand RNA viruses on the other hand, because their input genome directs translation, are thought to replicate exclusively in the cytoplasm. However, it has become increasingly apparent that many positive and negative strand RNA viruses whose primary site of replication is the cytoplasm use the nucleus and/or nuclear components in order to facilitate their replicative processes.

The nucleus of a mammalian cell contains the genetic information. The major nuclear functions reflect the need to transfer appropriate parts of this information to RNA (Jackson and Cook, 1995; Pombo et al., 2000). DNA is present in the form of chromosomes and these occupy discrete nuclear territories and preferred nuclear positions (Jackson and Cook, 1995; Fig. 1). However, the eukaryotic nucleus also contains a number of other domains or sub-compartments, which includes nucleoli (Lyon and Lamond, 2000), nuclear Cajal bodies (Olson et al., 2002), nuclear speckles (Fox et al., 2002), and transcription and replication foci (Lamond and Earnshaw, 1998). Prior to consideration of how viruses interact with the nucleus and sub-nuclear domains, the current state of knowledge of these structures will be outlined.

2. Sub-nuclear structures

The largest sub-nuclear structure, and perhaps the most studied, is the nucleolus (Fig. 2a). This structure is easily visible under the light microscope due to its high refractive index. For a number of years the principal
function of the nucleolus was thought to be ribosomal rRNA synthesis and ribosome biogenesis (Shaw and Jordan, 1995). Recently, however, the nucleolus has been implicated in many aspects of cell biology that include functions such as gene silencing, senescence, and cell cycle regulation (Carmo-Fonseca et al., 2000; Olson et al., 2000; Pederson, 1998; Scheer and Hock, 1999). Viral interactions with the nucleolus and its proteins have been found for DNA, RNA and retroviruses (Hiscox, 2002).

During interphase in higher eukaryotic cells the number of nucleoli varies depending on the stage of the cell cycle. The nucleolus disappears at the start of mitosis (Dundr et al., 2000) and during G1-phase cells can contain more than one nucleolus. This is probably reflected by the fact that these cells are translationally active, and therefore require increased ribosome production. A proteomic analysis of HeLa cell nucleoli concluded that they contain some 271 proteins (Andersen et al., 2002), including nucleolin, fibrillarin, and B23. Electron microscopy revealed that the nucleolus consists of at least three different regions; fibrillar centres, a dense fibrillar component and a granular component (Scheer and Hock, 1999). These regions may have different functions. For example, the peri-nucleolar compartment has been implicated in RNA metabolism (Huang et al., 1998).

Proteins are present in different domains of the nucleolus. Electron microscopy and immunofluorescence analysis showed that B23 is predominantly located in the granular region of the nucleolus, whereas nucleolin is largely present in the fibrillar centre and fibrillarin in the peri-nucleolar region. The concentration of nucleolar antigens, especially of B23 and nucleolin, appears to be controlled by the cell and depends on the physiological conditions. Nucleolin represents as much as 10% of total nucleolar protein and is highly phosphorylated, methylated, and can also be ADP-ribosylated (Ginisty et al., 1999). One of the main functions of nucleolin is processing the first cleavage step of ribosomal RNA (Ginisty et al., 1998) in the presence of U3 snoRNP (small nucleolar ribonucleoparticles). Other functions associated to be dependent on nucleolin are regulation of rDNA transcription, assembly of the nucleolus, as well as nucleocytoplasmic shuttling of proteins (Ginisty et al., 1999). During interphase and cytokinesis nucleolin is associated with B23 (Sirri et al., 1997).

Fibrillarin is the most abundant protein of both the dense fibrillar component and the fibrillar centre, but to
a lesser amount in the latter, but is absent from the granular component. Fibrillarin has a highly conserved structure of three domains, the central domain binding RNA (Aris and Blobel, 1991). Fibrillarin is crucial for nucleolar assembly at the end of telophase, the onset of rDNA transcription, the processing of rRNA and the splicing of snoRNA (Azum-Gelade et al., 1994; Fomproix et al., 1998). During mitosis, fibrillarin, as well as nucleolin and upstream binding factor (UBF) remain in pre-nucleolar bodies (PNB), which eventually localise to the nucleolar organising regions (NOR) at the end of mitosis.

Nucleolar proteins are also found in Cajal bodies, which vary in size and number depending on the cell type (Ogg and Lamond, 2002; Fig. 2b). They are predominately located at the periphery of the nucleolus, or even within the nucleolus itself (Platani et al., 2000), and the protein coilin probably mediates this interaction. The precise function of Cajal bodies has not been elucidated although they have been shown to contain factors required for transcription, splicing and ribosome biogenesis (Platani et al., 2002), and it is not unreasonable to hypothesise that Cajal bodies are involved in these processes (Ogg and Lamond, 2002). Cajal bodies have also been shown to sequester cell cycle regulatory complexes such as the CDK-2 cyclin E complex (Liu et al., 2000), and similar to the nucleolus, these structures may also play a role in regulation of the cell cycle.

3. Nuclear import and export

Molecules can enter the nucleus by passive diffusion or active transport mechanisms, depending on their size (Macara, 2001). Small molecules up to size of 50–60 kDa or less than 10 nm in diameter can diffuse passively through the nuclear pore complex (NPC), but most proteins are transported by energy driven transport mechanisms (Richardson et al., 1988).

Active transport of proteins is mediated by nuclear localisation signals (NLS). These signals are recognised by proteins of the importin super-family (importin α and β) that mediate the transport across the nuclear envelope using RanGTP (Macara, 2001).

NLSs were first identified in Simian Virus 40 large T antigen and from nucleoplasmin, and have subsequently been identified in a large number of proteins. Usually they contain short stretches of lysine or arginine residues, either as mono or bipartite signals. NLSs include the ‘pat4’ motif, which consists of a continuous stretch of four basic amino acids (arginine and lysine). The ‘pat7’ motif, which starts with a proline and is followed within three residues by a segment containing three basic residues out of four (Garcia-Bustos et al., 1991), or bipartite signals (Robbins et al., 1991).

Localisation of a protein to sub-nuclear structures like the nucleolus is probably a result of targeting to the nucleus via NLSs followed by an interaction between the target molecules (via a nucleolar localisation signal—that is in part an NLS) and components that make up the nucleolus (Carmo-Fonseca et al., 2000; Shaw and
Jordan, 1995). Whether proteins localise to the nucleolus or are retained there is uncertain. Certainly, general RNA binding proteins that are free to diffuse through the NPC might be predicted to localise to the nucleolus, where rRNA is being transcribed. In this case, such a protein would localise to the nucleoplasm and become concentrated in the nucleolus.

Polypeptides that contain NLSs are recognised and form complexes with importin α family in the perinuclear region. These complexes then associate with members of the importin β family, which localise the substrate to the central region of the NPC, where it passes through a gated channel. Once in the nucleoplasm, the complex disassembles and both importin α and -β are exported into the cytoplasm.

Exportins, as their name suggests, are molecules that facilitate transport of proteins/RNAs etc. out of the nucleus. Similar to NLSs, nuclear export signals (NESs) have been defined. One of the characteristic prototype signals is LxxLxxLxL, but other hydrophobic residues can substitute for several of the leucine residues; however, prolines situated between the hydrophobic residues can disrupt function (Bogerd et al., 1996). Many other NESs exist but do not conform to this particular motif (Macara, 2001). Leucine rich NESs are recognised by exportin CRM1/Xpo1. The study of Crm1 mediated pathways has benefited from the isolation of leptomycin B, an anti-fungal agent that specifically inhibits Crm1 function. In general Crm1 can export a wide variety of cargos, most of which contain an NES (Fornerod et al., 1997).

4. Localisation of viral proteins to the nucleus and nucleolus

In order to disrupt or usurp nuclear functions, RNA virus polypeptides can access the nucleus by appropriate pathways. Viral NLSs/NuLS can be identified by either sequence comparison to known sequences, or experimentally, where candidate motifs have been used to target fusion proteins (such as green fluorescent protein) to the nucleus or nucleolus (Fig. 3). One of the first descriptions of the nuclear localisation of a positive strand RNA virus protein was in the alphavirus, Semliki Forest virus (SFV). In this case both the SFV capsid (C) protein (Jakob, 1994, 1995; Michel et al., 1990) and nsP2 (Peranen et al., 1990) were observed to localise to the nucleus and/or nucleolus and found to contain NLSs that resembled cellular motifs. Indeed C protein was shown to contain two nucleolar targeting signals in the N-terminal region (Favre et al., 1994). The functional relevance of why these proteins would localise to the nucleus or nucleolus, and how this relates to their function in virus replication are both unknown. Non-structural protein nsP2 is involved in the regulation of minus strand RNA synthesis (Sawicki and Sawicki, 1993; Suopanki et al., 1998) and C protein is involved

![Fig. 3. Comparison of the intercellular localisation of the avian coronavirus infectious bronchitis virus nucleoprotein (N protein) that localises both to the cytoplasm and nucleolus (a) and a mutant protein that lacks a nucleolar localisation signal (b), which had been identified by sequence comparison to known nucleolar localisation signals (NuLS) (Hiscox et al., 2001). Vero cells were transfected with either a plasmid, pTriExIBVN that expressed a wild-type N protein fused to a C-terminal his-tag (Wurm et al., 2001) (a) or a plasmid, pTriExIBVNA351-372, in which the putative NuLS was deleted by overlapping PCR, no nucleolar localisation is observed (b). IBV N protein (red) and the his-tag (green) were detected by appropriate antibodies. Co-localisation, where it occurs, is yellow. Examples of cells in which N protein has localised to the nucleolus are arrowed. Magnification × 160.](image)
in nucleocapsid assembly and viral RNA binding (Owen and Kuhn, 1996; Weiss et al., 1989). However, C protein also associates with ribosomes to promote disassembly and assembly of the virus particle (Ulmanen et al., 1976; Wengler and Wengler, 1984). A conserved ribosome binding site (RBS) was identified in the C protein of alphaviruses (Wengler et al., 1992). C protein with a Mr of 33,000 may be expected to diffuse the NPC and localise to the nucleolus. However, Michel et al. (1990) showed that C protein accumulation in the nucleus was energy dependent, thus suggesting that transport across the NPC was active. C protein may localise preferentially to nucleoli via an interaction between the RBS and newly synthesised rRNA or ribosomal subunits. Although a recombinant SFV whose nsP2 contained altered NLS was reported to have identical properties to wild type virus (Rikkonen et al., 1994), recently Fazakerley et al. (2002) have reported that this change affects neurovirulence of SFV, and they speculated that this could be due to changes in processes involving RNA replication and/or the nuclear transport of nsP2.

Both the coronavirususes and arteriviruses show similar genome organisation (de Vries et al., 1997) and belong to the Nidovirales (Cavanagh, 1997). Although both families encode nucleoproteins (N proteins) whose principal function is to bind viral RNA, the proteins themselves are of a different size and have no discernable homology. However, in the case of several coronavirususes (Hiscox et al., 2001; Ning et al., 2003; Wurm et al., 2001) and arteriviruses (Rowland et al., 1999; Tijms et al., 2002), both N proteins localise to not only the cytoplasm, but also to the nucleolus in infected cells and cells expressing the N proteins alone.

The precise mechanism by which the coronavirus N protein localises to the nucleolus is unknown. However, similar to studies with the arterivirus porcine reproductive and respiratory syndrome virus N protein (Rowland et al., 1999), a GFP-tagged avian coronavirus N protein could localise to the nucleolus (Hiscox et al., 2001), and because the fusion protein was above the size exclusion limit of the NPC, indicated that N protein was actively transported into the nucleus. Rather than using nuclear import pathways directly, such proteins may ‘piggyback’ into the nucleus on other factors. For example, the coronavirus N protein has been shown co-localise with nucleolin in the nucleolus (Wurm et al., 2001), and bind to nucleolin via a protein:protein interaction (Chen et al., 2002), and thus may localise to the nucleolus because of its association with nucleolin.

Positive strand RNA virus proteins that localise to the nucleus would appear to have similar functions, i.e. binding to viral RNA (Table 1). One might predict that these proteins would localise to the nucleus if they are below the size exclusion limit of the NPC because they are arginine and lysine rich, and therefore, might associate with high concentrations of RNA, i.e. rRNA in the nucleolus, and therefore, this localisation plays no real part of the virus life cycle. However, there are several recent pieces of evidence to argue against this. First as discussed, some of these viral RNA binding proteins are actively transported into the nucleus. Second, and perhaps one of the key clues to a functional role of nuclear localisation, has been described by Tijms et al. (2002), who, using leptomycin B, demonstrated that the arterivirus N protein used the CRM-1 nuclear export pathway in order to shuttle from the nucleus to the cytoplasm, and that nuclear localisation of the protein was crucial for its function in virus assembly.

In the case of the mononegavirales, several of these viruses have proteins that localise to the nucleus or its periphery, and include viruses from the Paramyxoviridae and Rhabdoviridae (Table 1). For the paramyxoviruses examples include viral proteins from the genus Rubulavirus; human para-influenza virus type 2, and Newcastle disease virus (NDV), and genus Morbillivirus; measles virus and canine distemper virus (CDV). Human para-influenza virus type 2 V protein contains a NLS and localises to the nucleus (Watanabe et al., 1996) (but is also present in the cytoplasm (Nishio et al., 1999)). NDV M protein localises to the nucleus early in infection and becomes associated with nucleoli and remains in this structure throughout infection (Peeples et al., 1992). Studies with M protein of measles virus indicated that this protein controlled the accumulation of nucleocapsids in the cytoplasm and nucleus (Patterson et al., 2001). CDV nucleocapsid protein localises to the nucleus with the signals for this contained within the N-terminal region (Yoshida et al., 1999). The Rhabdovirus vesicular stomatitis virus (VSV) matrix (M) protein associates with the nuclear rim of the NPC and inhibits nucleoporin 98 (Nup98) dependent nuclear transport (Enninga et al., 2002). Indeed VSV M protein, although smaller than the size exclusion limits for transit through the NPC, is actively imported into the nucleus and was shown to contain two separate NLSs (Glodowski et al., 2002). Surprisingly, VSV G protein localises to the nucleus, and Da Poian et al. (1996) attributed this to the fact that uncoating of the viral RNA may occur in close proximity to the nuclear membrane.

5. The redistribution of nuclear proteins and their association with virus during infection

Although cytoplasmic RNA viruses confine their principal replicative functions to membrane bound structures in the cytoplasm (Gosert et al., 2002; Kujala et al., 2001; Lyle et al., 2002), many of these viruses may use proteins associated with nuclear functions in order to facilitate replication or sequester such factors to disrupt nuclear functions. One mechanism by which
viruses can achieve this is by disruption of nucleocytoplasmic trafficking, which may redistribute proteins that would other localise in the nucleus to the cytoplasm. Several picornaviruses and also VSV have been shown to alter nucleo-cytoplasmic trafficking (Belov et al., 2000).

Poliovirus infection results in the re-localisation of certain nuclear proteins by blocking nuclear import pathways, concomitant with the degradation of specific proteins of the NPC (Gustin and Sarnow, 2001). This has also been seen in rhinovirus-infected cells in which proteins involved in nuclear shuttling accumulate at the cytoplasmic side of the NPC. The observation was attributed to the degradation of nucleoporins Nup153 and p62 (Gustin and Sarnow, 2002). The M protein of VSV and related viruses associates with the nuclear rim of the NPC and inhibits nuclear import and exit (Enninga et al., 2002; von Kobbe et al., 2000). VSV leader RNA binds heterogeneous nuclear ribonucleoprotein particle U (hnRNP U), which is involved in pre-mRNA processing, and may have a similar role in VSV replication (Gupta et al., 1998). Gustin and Sarnow (2002) suggested that the redistribution of nuclear proteins and disruption of the NPC might be part of a strategy by which cytoplasmic RNA viruses could avoid triggering the host immune response by blocking nuclear signalling pathways. Certainly Enninga et al. (2002) have shown that VSV M protein targets the NPC component Nup98, as part of a strategy to disrupt an interferon mediated response.

Several nuclear factors have been implicated in the regulation of translation directed by internal ribosome entry sites (IRES) present at the 5' end of the picornavirus, pestivirus and flavivirus genomes, and may explain why picornaviruses disrupt nuclear–cytoplasmic trafficking. One of these factors is the La protein, an

Table 1
Examples of nuclear involvement of cytoplasmic RNA virus proteins

| Virus                     | Viral protein | Viral associated function                                      | Nuclear effect                                | Reference (nuclear effect) |
|---------------------------|---------------|-----------------------------------------------------------------|-----------------------------------------------|---------------------------|
| Nidovirales               |               |                                                                 |                                               |                           |
| Coronaviruses (IBV, MHV and TGEV) | Nucleoprotein | Binds to viral RNA to form part of virus core, possible other roles in virus replication and host cell interactions | Localises to nucleolus, associates with nucleolin and redistributes fibrillarin | (Chen et al., 2002; Hiscox et al., 2001; Wurm et al., 2001) |
| Arteriviruses             |               |                                                                 |                                               |                           |
| PRRSV                    | Nucleocapsid  | Binds to viral RNA to form part of virus core, Binds to viral RNA to form part of virus core, | Localises to the nucleolus, Localises to the nucleus | (Rowland et al., 1999) |
| Equine arteritis virus    | Nucleocapsid  | Binds to viral RNA to form part of virus core, has to shuttle to the nucleus and back out to the cytoplasm |                                               | (Tijms et al., 2002)    |
|                          | Nsp1          | Transcription of subgenomic mRNAs                               | Localises to the nucleus                      |                           |
| Flaviviridae              |               |                                                                 |                                               |                           |
| Flavivirus                | Dengue virus  | Core Binds to viral RNA to form ribonucleocapsid                | Localises to the nucleus and nucleolus        | (Wang et al., 2002)      |
| Hepatitis C virus         | NS5B          | RNA dependent RNA polymerase                                    | Redistributes nucleolin                       | (Hirano et al., 2003)    |
|                           | Core          | Binds to viral RNA                                              | Affects p21 expression                        | (Yamanaka et al., 2002)  |
| Togaviridae               |               |                                                                 |                                               |                           |
| Alphavirus                | Capsid        | Nucleocapsid assembly and viral RNA binding                    | Localises to the nucleolus                    | (Jakob, 1994; Michel et al., 1990) |
|                           | nsP2          | Regulation of minus strand RNA synthesis and involved in neuro-virulence | Transported to the nucleus                    | (Peranen et al., 1990)   |
| Mononegavirales           |               |                                                                 |                                               |                           |
| Paramyxoviridae           | V protein     | Causes rapid degradation of STAT2 protein                      | Localises to the nucleus                      | (Watanabe et al., 1996)  |
| Human parainfluenza virus type 2 | NDV | M protein | Controlled the accumulation of nucleocapsids in the cytoplasm and nucleus | Localises to the nucleolus | (Patterson et al., 2001) |
|                           | M protein     | Controlled the accumulation of nucleocapsids in the cytoplasm and nucleus | Localises to the nucleolus                    | (Peeples et al., 1992)   |
| Rhabdoviridae             | VSV           | Matrix Blocks STAT activation                                   | Inhibits Nup98 dependent nuclear transport    | (Enninga et al., 2002; Glodowski et al., 2002) |
RNA binding protein predominately located in the nucleus and involved in initiation and termination of RNA polymerase III transcription (Wolin and Cedervall, 2002). La protein was shown to enhance the translation of several viral genomes, including poliovirus and HCV (Belsham et al., 1995). However, in the case of HCV, La is required at lower concentration (Isoyama et al., 1999) than with poliovirus. As a possible mechanism to control the amount of La protein, picornavirus infection results in the redistribution of La from the nucleus to the cytoplasm, whereas this does not occur in HCV infected cells (Isoyama et al., 1999). In poliovirus infected cells La protein was shown to be C-terminal cleaved, possibly by the 3C protease (Shiroki et al., 1999). Green fluorescent protein linked to the C-terminal region of La demonstrated that this region was involved in nuclear localisation. The N-terminal region of La localised to the cytoplasm and retained the ability to enhance IRES dependent translation of the poliovirus genome (Shiroki et al., 1999).

Polypyrimidine tract binding protein (PTB, also known as p57 and hnRNP1) shuttles between the nucleus and cytoplasm in a transcription dependent manner, contains a NLS, and has been proposed as a splicing factor (Patton et al., 1991). PTB also interacts with the IRESs of several picornaviruses (Belsham and Sonenberg, 2000). During poliovirus infection cellular transcription is inhibited and PTB was shown to redistributed to the cytoplasm (Back et al., 2002). In addition to having the ability to cleave La, the 3C protease also cleaves PTB, and it is these forms that are redistributed from the nucleus to the cytoplasm. This may contribute to a switch from translation to replication of the poliovirus genome (Back et al., 2002). PTB has also been shown to interact with the coronavirus genome (Huang and Lai, 1999; Li et al., 1999) and has been shown to affect the coronavirus murine hepatitis virus transcription (Choi et al., 2002). hnRNP A1 was found to associate with the coronavirus genome and N protein (Wang and Zhang, 1999; Zhang et al., 1999), and was postulated to be involved in virus transcription and replication (Shi et al., 2000). However, subsequent genetic studies indicated that it played no role in these processes (Shen and Masters, 2001).

Several other nuclear proteins have been described which interact with poliovirus. Once such protein, identified by a yeast two hybrid screen is Sam68 (McBride et al., 1996), a protein that associates with Src during mitosis (Guitard et al., 1998). During poliovirus infection this protein localises from the nucleus to the cytoplasm and associates with the viral protein 2C (McBride et al., 1996), which is involved in membrane binding (Aldabe and Carrasco, 1995) and RNA binding (Rodriguez and Carrasco, 1995) and has ATPase activity (Mirzayan and Wimmer, 1994). Sam68 has also been implicated in cell cycle control by modulating RNA metabolism, indeed Li et al. (2002) suggested that disruption of Sam68 may play a role in the G2 to M phase progression. Although it is likely that Sam68 is recruited by the 2C polymerase for its RNA binding function, by altering the distribution of Sam68 during infection, picornaviruses may also disrupt the cell cycle.

Nucleolin is prevented from entering the nucleus in poliovirus infected cells, and has been shown to interact with the poliovirus 3′ non-coding region (NCR). As a result, it has been suggested to be involved in virus replication (Waggoner and Sarnow, 1998). Izumi et al. (2001) demonstrated that nucleolin bound to the 5′ UTR sequence on the poliovirus genome and stimulated IRES dependent translation. Interestingly, nucleolin (and proteins belonging to the nucleolin super-family) have also been suggested to act as a possible cell surface receptor for coxsackie B viruses (Raab de Verdugo et al., 1995). Another nucleolar protein, fibrillarin, is redistributed in coronavirus infected cells and cells transiently expressing the coronavirus N protein (Chen et al., 2002).

6. Viral interference with nuclear functions

One of the most obvious effects of virus infection is the induction of the interferon and cytoplasmic RNA viruses have a number of strategies to combat this response (and have recently been reviewed; (Goodbourn et al., 2000; Katze et al., 2002; Young et al., 2000)). For example, non-cytopathogenic bovine viral diarrhoea virus (BVDV) infection results in the failure of cells to produce either interferon α/β, possibly due to inhibition of interferon regulatory factor 3 function (Baigent et al., 2002) and both Sendai virus and simian virus 5 block the activation of interferon (Didcock et al., 1999).

Several cytoplasmic RNA viruses interfere with other host cell nuclear functions such as cell cycle control (Feuer et al., 2002) and transcription. Poliovirus protease 3C is responsible for the shutoff of Pol I transcription in infected cells (Rubinstein et al., 1992). Poliovirus also shuts down host cell transcription in neighbouring uninfected cells, possibly through accumulation of poliovirus proteins in host cell nuclei (Bossart et al., 1984). Thus viruses may pre-program uninfected cells prior to virus infection in order to promote favourable metabolic conditions for virus replication.

VSV also interferes with cellular transcription. The VSV plus strand leader RNA localises to the nucleus (Kurilla et al., 1982) and can inhibit DNA dependent transcription (McGowan et al., 1982; Remenick et al., 1988). However, in VSV infected cells the primary cause of decreased cellular transcription is host cell shut off associated with the M protein (Black et al., 1993). The VSV leader RNA has been shown to bind La (Kurilla et al., 1982).
7. Conclusion

Interaction of viruses with the nucleus, nuclear subdomains and proteins does not appear to be restricted to those viruses that use the nucleus as a site of replication. Many positive and negative strand RNA viruses whose primary site of replication is the cytoplasm sequester nuclear factors in order to facilitate virus replication and, by altering nuclear–cytoplasmic trafficking, disrupt host cell functions and cellular responses to viral infections. Both successful replication and avoiding the host response to infection are a prerequisite for the successful evolutionary persistence of a virus.

Acknowledgements

The author would like to thank Dr John McCauley and Dr Sean Whelan for their critical reading of this manuscript and helpful suggestions. The author’s own research is supported by the BBSRC (45/S12883).

References

Aldebe, R., Carrasco, L. 1995. Induction of membrane proliferation by poliovirus proteins 2C and 2BC. Biochem. Biophys. Res. Commun. 206, 64–76.

Andersen, J.S., Lyon, C.E., Fox, A.H., Leung, A.K.L., Lam, Y.W., Steen, H., Mann, M., Lamond, A.I. 2002. Directed proteomic analysis of the human nucleolus. Curr. Biol. 12, 1–11.

Aris, J.P., Blobel, G. 1991. cDNA cloning and sequencing of human fibrillarin, a conserved nucleolar protein recognised by autoimmune antisera. Proc. Natl. Acad. Sci. USA 88, 931–935.

Azum-Gelade, M.-C., Noaillac-Depeyre, J., Caizergues-Ferrer, M., Gas, N., 1994. Cycle cell redistribution of U3 snRNA and fibrillarin. J. Cell Sci. 107, 463–475.

Back, S.H., Kim, Y.K., Kim, W.J., Cho, S., Oh, H.R., Kim, J.E., Jang, S.K. 2002. Translation of polioviral mRNA is inhibited by cleavage of polypyrimidine tract-binding proteins executed by polioviral 3C(pro). J. Virol. 76, 2529–2542.

Baigent, S.J., Zhang, G., Fray, M.D., Flick-Smith, H., Goodbourn, S., McCauley, J.W. 2002. Inhibition of beta interferon transcription by noncytopathogenic bovine viral diarrhoea virus is through an interferon regulatory factor 3-dependent mechanism. J. Virol. 76, 8979–8988.

Belsham, G.J., Sonenberg, N. 2000. Picornavirus RNA translation: roles for cellular proteins. Trends Microbiol. 8, 330–335.

Belsham, G.J., Sonenberg, N., Svitkin, Y.V. 1995. The role of the La autoantigen in internal initiation. Curr. Top. Microbiol. Immunol. 203, 85–98.

Black, B.L., Rhodes, R.B., McKenzie, M., Lyles, D.S. 1993. The role of vesicular stomatitis virus matrix protein in inhibition of host-directed gene expression is genetically separable from its function in virus assembly. J. Virol. 67, 4814–4821.

Bogerd, H.P., Friddell, R.A., Benson, R.E., Hua, J., Cullen, B.R. 1996. Protein sequence requirements for function of the human T-cell leukemia virus type 1 Rex nuclear export signal delineated by a novel in vivo randomization-selection assay. Mol. Cell Biol. 16, 4207–4214.

Bosser, W., Egger, D., Rasser, Y., Bienz, K. 1984. Accumulation of poliovirus proteins in uninfected HEP-2 cell nuclei in vitro. Intervirology 21, 150–158.

Carmo-Fonseca, M., Mendes-Soares, L., Campos, I. 2000. To be or not to be in the nucleolus. Nat. Cell Biol. 2, 107–112.

Cavanagh, D., 1997. Nidovirales: a new order comprising Coronaviridae and Arteriviridae. Arch. Virol. 140, 629–633.

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

Choi, K.S., Huang, P., Lai, M.M. 2002. Polypyrimidine-tract-binding protein affects transcription but not translation of mouse hepatitis virus RNA. Virology 303, 58–68.

Da Poian, A.T., Gomes, A.M., Oliveira, R.J., Silva, J.L. 1996. Migration of vesicular stomatitis virus glycoprotein to the nucleus of infected cells. Proc. Natl. Acad. Sci. USA 93, 8268–8273.

de Vries, A.A.F., Horzinek, M.C., Rottier, P.J.M., de Groot, R.J. 1997. The genome organisation of the nidovirales: similarities and differences between arteri-, toro-, and coronaviruses. Sem. Virol. 8, 33–47.

Didcock, L., Young, D.F., Goodbourn, S., Randall, R.E. 1999. Sendai virus and simian virus 5 block activation of interferon-responsive genes: importance for virus pathogenesis. J. Virol. 73, 3125–3133.

Dundr, M., Misteli, T., Olson, M.O.J. 2000. The dynamics of postmitotic reassembly of the nucleolus. J. Cell Biol. 150, 433–446.

Enninga, J., Levy, D.E., Blobel, G., Fontoura, B.M. 2002. Role of nucleoporin induction in releasing an mRNA nuclear export block. Science 295, 1523–1525.

Favre, D., Studer, E., Michel, M.R. 1994. Two nucleolar targeting signals present in the N-terminal part of Semliki Forest virus capsid protein. Arch. Virol. 137, 149–155.

Fazakerley, J.K., Boyd, A., Mikkola, M.L., Kaariainen, L. 2002. A single amino acid change in the nuclear localisation sequence of the nsp2 protein affects the neurovirulence of semliki forest virus. J. Virol. 76, 392–396.

Feuer, R., Mena, I., Pagarigan, R., Silfa, M.K., Whitto, J.L. 2002. Cell cycle status affects coxsackievirus replication, persistence, and reactivation in vitro. J. Virol. 76, 4430–4440.

Fomproix, N., Gebran-Younes, J., Hernandez-Verdun, D. 1998. Effects of anti-fibrillarin antibodies on building of functional nucleoli at the end of mitosis. J. Cell Sci. 111, 359–372.

Fornerod, M., Ohno, M., Yoshida, M., Mattaj, I.W. 1997. CRM1 is an export receptor for leucine-rich nuclear export signals. Cell 90, 1051–1060.

Fox, A.H., Lam, Y.W., Leung, A.K., Lyon, C.E., Andersen, J., Mann, M., Lamond, A.I. 2002. Paraspeckles: a novel nuclear domain. Curr. Biol. 12, 13–25.

Garcia-Bustos, J., Heitman, J., Hall, M.N. 1991. Nuclear protein localisation. Biochem. Biochim. Biophys. Acta 1071, 83–101.

Ginisty, H., Amaelric, F., Bouvet, P. 1998. Nucleolin functions in the first step of ribosomal RNA processing. EMBO J. 17, 1476–1486.

Ginisty, H., Sicard, H., Roger, B., Bouvet, P. 1999. Structure and functions of nucleolin. J. Cell Sci. 112, 761–772.

Gladowski, D.R., Petersen, J.M., Dahlberg, J.E. 2002. Complex nuclear localisation signals in the matrix protein of vesicular stomatitis virus. J. Biol. Chem. 277, 46864–46870.

Goodbourn, S., Didcock, L., Randall, R.E. 2000. Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. J. Gen. Virol. 81, 2341–2364.
Gosert, R., Kanjanahaluthai, A., Egger, D., Bienz, K., Baker, S.C., 2002. RNA replication of mouse hepatitis virus takes place at double-membrane vesicles. J. Virol. 76, 3697–3708.

Guittard, E., Barlat, I., Maurier, F., Schweighoffer, F., Toque, B., 1998. Sam68 is a Ras-GAP-associated protein in mitosis. Biochem. Biophys. Res. Commun. 245, 562–566.

Gupta, A.K., Drazba, J.A., Banerjee, A.K., 1998. Specific interaction of heterogeneous nuclear ribonucleoprotein particle U with the leader RNA sequence of vesicular stomatitis virus. J. Virol. 72, 8532–8540.

Gustin, K.E., Sarnow, P., 2001. Effects of poliovirus infection on nucleo-cytoplasmic trafficking and nuclear pore complex formation. EMBO J. 20, 240–249.

Gustin, K.E., Sarnow, P., 2002. Inhibition of nuclear import and alteration of nuclear pore complex composition by rhinovirus. J. Virol. 76, 8787–8796.

Hirano, M., Kaneko, S., Yamashita, T., Luo, H., Qin, W., Shirota, Y., Nomura, T., Kobayashi, K., Murakami, S., 2003. Direct interaction between nucleolin and hepatitis C virus NS5B. J. Biol. Chem. 278, 5109–5115.

Hiscox, J.A., 2002. Brief review: the nucleolus—a gateway to viral infection. Arch. Virol. 147, 1077–1089.

Hiscox, J.A., Wurm, T., Wilson, L., Cavanagh, D., Britton, P., Brooks, G., 2001. The coronavirus infectious bronchitis virus nucleoprotein localises to the nucleolus. J. Virol. 75, 506–512.

Huang, P.Y., Lai, M.M.C., 1999. Polypyrimidine tract-binding protein binds to the leader RNA of mouse hepatitis virus NS5B. J. Cell Biol. 143, 35–47.

Isoyama, T., Kamoshita, N., Yasui, K., Iwai, A., Shiroki, K., Toyoda, H., Yamada, A., Takasay, Y., Nomoto, A., 1999. Lower concentration of La protein required for internal ribosome entry on hepatitis C virus RNA than on poliovirus RNA. J. Virol. 73, 2333–2337.

Izumi, R.E., Valdez, B., Banerjee, R., Srivastava, M., Dasgupta, A., 2001. Nucleolin stimulates viral internal ribosome entry site-mediated translation. Virus Res. 76, 17–29.

Jackson, D.A., Cook, P.R., 1995. The structural basis of nuclear function. Int. Rev. Cytol. 162A, 125–149.

Jakob, R., 1994. Nuclear accumulation of Semliki Forest virus nucleocapsid C protein: influence of metabolic status, cytoskeleton and receptors. J. Med. Microbiol. 40, 389–392.

Jakob, R., 1995. Electroporation-mediated delivery of nucleolar targeting sequences from Semliki Forest virus nucleocapsid protein. Prep. Biochem. 25, 99–117.

Katze, M.G., He, Y., Gale, M., Jr, 2002. Viruses and interferon: a fight for supremacy. Nat. Rev. Immunol. 2, 675–687.

Kujala, P., Ikaheimonen, A., Ehsani, N., Vihinen, H., Auvinen, P., Kaariainen, L., 2001. Biogenesis of the Semliki Forest virus RNA replication complex. J. Virol. 75, 3873–3884.

Kurilla, M.G., Keene, J.D., 1983. The leader RNA of vesicular stomatitis virus is bound by a cellular protein reactive with anti-Lu lupus antibodies. Cell 34, 837–845.

Kurilla, M.G., Pwnica-Worms, H., Keene, J.D., 1982. Rapid and transient localisation of the leader RNA of vesicular stomatitis virus in the nuclei of infected cells. Proc. Natl. Acad. Sci. USA 79, 5240–5244.

Lamond, A.I., Earnshaw, W.C., 1998. Structure and function in the nucleus. Science 280, 547–553.

Li, H.P., Huang, P.Y., Park, S.M., Lai, M.M.C., 1999. Polypyrimidine tract-binding protein binds to the leader RNA of mouse hepatitis virus and serves as a regulator of viral transcription. J. Virol. 73, 772–777.

Li, Q.H., Haga, I., Shimizu, T., Itoh, M., Kurosaki, T., Fujisawa, J., 2002. Retardation of the G2-M phase progression on gene disruption of RNA binding protein Sam68 in the DT40 cell line. FEBS Lett. 525, 145–150.

Liu, J.-L., Hebert, M.B., Ye, Y., Templeton, D.J., King, H.-J., Matera, A.G., 2000. Cell cycle-dependent localisation of the Cdk2-cyclin E complex in Cajal (coiled) bodies. J. Cell Sci. 113, 1543–1552.

Lyle, J.M., Bullitt, E., Bienz, K., Kirkegaard, K., 2002. Visualization and functional analysis of RNA-dependent RNA polymerase lattices. Science 296, 2218–2222.

Lyons, C.E., Lamond, A.I., 2000. The nucleolus. Curr. Biol. 10, 323, 324. Macara, I.G., 2001. Transport into and out of the nucleus. Microbiol. Mol. Biol. Rev. 65, 570–594.

McBride, A.E., Schlegel, A., Kirkegaard, K., 1996. Human protein Sam68 relocation and interaction with poliovirus RNA polymerase in infected cells. Proc. Natl. Acad. Sci. USA 93, 2296–2301.

McGowan, J.J., Emerson, S.U., Wagner, R.R., 1982. The plus-strand leader RNA of VSV inhibits DNA-dependent transcription of adenovirus and SV40 genes in a soluble whole-cell extract. Cell 28, 325–333.

Michel, M.R., Elgizoli, M., Dai, Y., Jakob, R., Koble, H., Arrigo, A.P., 1990. Karyophilic properties of Semliki Forest virus nucleocapsid protein. J. Virol. 64, 5123–5131.

Mirzayan, C., Wimmer, E., 1994. Biochemical studies on poliovirus polypeptide 2C: evidence for ATPase activity. Virology 199, 176–187.

Ning, Q., Lakatoo, S., Liu, M.F., Yang, W.M., Wang, Z.M., Phillips, M.J., Levy, G.A., 2003. Induction of prothrombinase fg2 by the nucleocapsid protein of virulent mouse hepatitis virus is dependent on host hepatic nuclear factor-4 alpha. J. Biol. Chem. 278, 15541–15549.

Nishio, M., Tsurudome, M., Ito, M., Kawano, M., Kusagawa, S., Komada, H., Ito, Y., 1999. Isolation of monoclonal antibodies directed against the V protein of human parainfluenza virus type 2 and localisation of the V protein in virus-infected cells. Med. Microbiol. Immunol. 188, 79–82.

Ogg, S.C., Lamond, A.I., 2002. Cajal bodies and colin-moving towards function. J. Cell Biol. 159, 17–21.

Olson, M.O., Dundr, M., Szebeni, A., 2000. The nucleolus: an old factory with unexpected capabilities. Trends Cell Biol. 10, 189–196.

Olson, M.O., Hingorani, K., Szebeni, A., 2002. Conventional and nonconventional roles of the nucleolus. Int. Rev. Cytol. 219, 199–266.

Owen, K.E., Kuhn, R.J., 1996. Identification of a region in the Sindbis virus nucleocapsid protein that is involved in specificity of RNA encapsidation. J. Virol. 70, 2757–2763.

Patterson, J.B., Cornu, T.I., Redwine, J., Dales, S., Lewicki, H., Holz, A., Thomas, D., Billeter, M.A., Oldstone, M.B., 2001. Evidence that the hypermutated M protein of a subacute sclerosing panencephalitis measles virus actively contributes to the chronic progressive CNS disease. Virology 291, 215–225.

Patton, J.G., Mayer, S.A., Tempst, P., Nadal-Ginard, B., 1991. Characterization and molecular cloning of polypyrimidine tract-binding protein: a component of a complex necessary for pre-mRNA splicing. Gene Dev. 5, 1237–1251.

Pederson, T., 1998. The plurifunctional nucleolus. Nucleic Acids Res. 26, 3871–3876.

Peebles, M.E., Wang, C., Gupta, K.C., Coleman, N., 1992. Nuclear entry and nucleolar localisation of the Newcastle disease virus (NDV) matrix protein occur early in infection and do not require other NDV proteins. J. Virol. 66, 3263–3269.

Peranen, J., Rikkonen, M., Liljestrom, P., Kaariainen, L., 1990. Nuclear localisation of Semliki Forest virus-specific nonstructural protein nsP2. J. Virol. 64, 1888–1896.
Platani, M., Goldberg, I., Swedlow, J.R., Lamond, A.I., 2000. In vivo analysis of Cajal body movement, separation, and joining in live human cells. J. Cell Biol. 151, 1561–1574.

Platani, M., Goldberg, I., Lamond, A.I., Swedlow, J.R., 2002. Cajal body dynamics and association with chromatin are ATP-dependent. Nat. Cell Biol. 4, 502–508.

Pombo, A., Jones, E., Iborra, F.J., Kimura, H., Sugaya, K., Cook, P.R., Jackson, D.A., 2000. Specialized transcription factories within mammalian nuclei. Crit. Rev. Eukaryot. Gene Expr. 10, 21–29.

Raab de Verdugo, U., Selinka, H.-C., Huber, M., Kramer, B., Kellermann, J., Hofschneider, P.H., Kandolf, R., 1995. Characterization of a 100-kDa binding protein for the six serotypes of coxsackie B viruses. J. Virol. 69, 6751–6757.

Remenick, J., Kenny, M.K., McGowan, J.J., 1988. Inhibition of adenovirus DNA replication by vesicular stomatitis virus leader RNA. J. Virol. 62, 1286–1292.

Richardson, W.D., Mills, A.D., Dilworth, S.M., Laskey, R.A., Dingwall, C., 1988. Nuclear protein migration involves two steps: rapid binding at the nuclear envelope followed by slower translocation through nuclear pores. Cell 52, 655–664.

Rikkonen, M., Peranen, J., Kaariainen, L., 1994. Nuclear targeting of Semliki Forest virus nsP2. Arch. Virol. 9 (Suppl.), 369–377.

Robbins, J., Dilworth, S.M., Laskey, R.A., Dingwall, C., 1991. Two interdependent basic domains in nucleoplasmic nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. Cell 64, 615–623.

Rodriguez, P.L., Carasco, L., 1995. Poliovirus protein 2C contains two regions involved in RNA binding activity. J. Biol. Chem. 270, 10105–10112.

Rowland, R.R., Kerwin, R., Kuckleburg, C., Sperlich, A., Benfield, D.A., 1999. The localisation of porcine reproductive and respiratory syndrome virus nucleocapsid protein to the nucleus of infected cells and identification of a potential nucleolar localisation signal sequence. Virus Res. 64, 1–12.

Rubinstein, S.J., Hammerle, T., Wimmer, E., Dasgupta, A., 1992. Infection of HeLa cells with poliovirus results in modification of a complex that binds to the RNA promoter. J. Virol. 66, 3062–3068.

Sawicki, D.L., Sawicki, S.G., 1993. A second nonstructural protein component nsP2 of poliovirus. J. Gen. Virol. 79, 309–319.

Tijms, M.A., van der Meer, Y., Snijder, E.J., 2002. Nuclear localisation of non-structural protein 1 and nucleocapsid protein of equine arteritis virus. J. Gen. Virol. 83, 795–800.

Ulmann, I., Soderlund, H., Kaariainen, L., 1976. Semliki forest virus capsid protein associates with 60S ribosomal subunit in infected cells. J. Virol. 20, 203–210.

Von Kobbe, C., van Deursen, J.M., Rodrigue, J.P., Sitterlin, D., Bachi, A., Wu, X., Wilm, M., Carmona-Fonseca, M., Izaurrelde, E., 2000. Vesicular stomatitis virus matrix protein inhibits host cell gene expression by targeting the nucleoporin Nup98. Mol. Cell 6, 1243–1252.

Waggoner, S., Sarnow, P., 1998. Viral ribonucleoprotein complex formation and nucleolar-cytoplasmic relocalization of nucleolin in poliovirus-infected cells. J. Virol. 72, 6699–6709.

Wang, Y.C., Zhang, X.M., 1999. The nucleocapsid protein of coronavirus mouse hepatitis virus interacts with the cellular heterogeneous nucleoplasmic protein A1 as a pre-receptor. J. Virol. 63, 96–109.

Wang, S.H., Xu, W.J., Huang, K.J., Lei, H.Y., Yao, C.W., King, C.C., Hu, S.T., 2002. Intracellular localisation and determination of a nuclear localisation signal of the core protein of Dengue virus. J. Gen. Virol. 83, 3093–3102.

Watanabe, N., Kawano, M., Tsurdome, M., Kusagawa, S., Nishio, M., Komada, H., Shima, T., Ito, Y., 1996. Identification of the sequences responsible for nuclear targeting of the V protein of human parainfluenza virus type 2. J. Gen. Virol. 77, 327–338.

Whittaker, G.R., Kann, M., Helenius, A., 2000. Viral entry into the nucleus. Annu. Rev. Cell Dev. Biol. 16, 627–651.

Wols, S.L., Cedervall, T., 2002. The la protein. Annu. Rev. Biochem. 71, 375–403.

Wurm, T., Chen, H., Britton, P., Brooks, G., Hiscox, J.A., 2001. Localization of the la protein to the nuclear envelope in infected cells. J. Virol. 75, 9345–9356.

Watanabe, N., Kawano, M., Tsurdome, M., Kusagawa, S., Nishio, M., Komada, H., Shima, T., Ito, Y., 1996. Identification of the sequences responsible for nuclear targeting of the V protein of human parainfluenza virus type 2. J. Gen. Virol. 77, 327–338.

Wassil, J., Kurilla, M.G., Keene, J.D., 1983. A host protein (La) binds to a unique species of minus-sense leader RNA during replication of vesicular stomatitis virus. Proc. Natl. Acad. Sci. USA 80, 5827–5831.

Wool, S.L., Cedervall, T., 2002. The la protein. Annu. Rev. Biochem. 71, 375–403.

Wurm, T., Chen, H., Britton, P., Brooks, G., Hiscox, J.A., 2001. Localization of the la protein to the nuclear envelope in infected cells. J. Virol. 75, 9345–9356.

Yamanaka, T., Kodama, T., Doi, T., 2002. Subcellular localisation of HCV core protein which mediates interaction of cores with ribosomes and the disassembly of cores. Virology 191, 880–888.

Whittaker, G.R., Kann, M., Helenius, A., 2000. Viral entry into the nucleus. Annu. Rev. Cell Dev. Biol. 16, 627–651.

Wolus, J., Cervall, T., 2002. The la protein. Annu. Rev. Biochem. 71, 375–403.

Young, D.F., Didcock, L., Goodburn, S., Randall, R.E., 2000. Paramyxoviridae use distinct virus-specific mechanisms to circumvent the interferon response. Virology 269, 383–390.

Zhang, X.M., Li, H.P., Xue, W.M., Lai, M.M.C., 1999. Formation of a ribonucleoprotein complex of mouse hepatitis virus involving heterogeneous nuclear ribonucleoprotein A1 and transcription-regulatory elements of viral RNA. Virology 264, 115–124.