Virology 452-453 (2014) 212–222

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

The nucleolar phosphoprotein B23 targets Newcastle disease virus matrix protein to the nucleoli and facilitates viral replication

Zhiqiang Duan [a,c], Jian Chen [a], Haixu Xu [a], Jie Zhu [a], Qunhui Li [a], Liang He [a], Huimou Liu [a,c], Shunlin Hu [a,b,c], Xiufan Liu [a,b,c,*]

[a] Animal Infectious Disease Laboratory, College of Veterinary Medicine, Yangzhou University, Yangzhou 225009, China
[b] Jiangsu Co-innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou 225009, China
[c] Ministry of Educational Key Lab for Avian Preventive Medicine, Yangzhou University, Yangzhou 225009, China

ARTICLE INFO

Article history:
Received 15 October 2013
Returned to author for revisions
28 November 2013
Accepted 17 January 2014
Available online 10 February 2014

Keywords:
Newcastle disease virus
Matrix protein
Nucleolar protein
B23
Viral replication

ABSTRACT

The cellular nucleolar proteins are reported to facilitate the replication cycles of some human and animal viruses by interaction with viral proteins. In this study, a nucleolar phosphoprotein B23 was identified to interact with Newcastle disease virus (NDV) matrix (M) protein. We found that NDV M protein accumulated in the nucleolus by binding B23 early in infection, but resulted in the redistribution of B23 from the nucleoli to the nucleoplasm later in infection. *In vitro* binding studies utilizing deletion mutants indicated that amino acids 30–60 of M and amino acids 188–245 of B23 were required for binding. Furthermore, knockdown of B23 by siRNA or overexpression of B23 or M-binding B23-derived polypeptides remarkably reduced cytopathic effect and inhibited NDV replication. Collectively, we show that B23 facilitates NDV replication by targeting M to the nucleolus, demonstrating for the first time a direct role for nucleolar protein B23 in a paramyxovirus replication process.

Introduction

Newcastle disease virus (NDV), a member of the *Paramyxoviridae* family, is an enveloped virus with a negative-sense single-stranded RNA genome that encodes at least six proteins in the order 3′-NP–P–M–F–HN–L-5′ (Alexander, 2000). Of all these structural proteins, the matrix (M) protein is the most abundant protein in virions and forms an outer protein shell around the nucleocapsid, constituting the bridge between the viral envelope and the nucleocapsid (Battisti et al., 2012; Yusoff and Tan, 2001). Like M proteins of other paramyxoviruses, the NDV M protein is demonstrated to be a nucleocytoplasmic shuttling protein and plays key roles in NDV replication cycle (Harrison et al., 2010). In addition to functioning for the assembly and budding of viral particles in the cytoplasm (Pantua et al., 2006), the NDV M protein is observed to localize in the nucleus early in infection and becomes associated with nucleoli and remains in this structure throughout infection (Duan et al., 2013a, 2013b; Peeples, 1988; Peeples et al., 1992). This nuclear-nucleolar localization of M protein is thought to ensure that viral replication and transcription in the cytoplasm proceed smoothly, which is by analogy with the measles virus M protein (Iwasaki et al., 2009), and also inhibit host RNA and protein synthesis similar to the vesicular stomatitis virus M protein (Rajani et al., 2012; Yuan et al., 2001). Up to now, the only paramyxovirus M proteins observed in the nucleus and the nucleolus have been that of Sendai virus (Yoshida et al., 1979) and NDV (Peeples et al., 1992). Previous study has indicated that NDV M protein enters the nucleus via a bipartite nuclear localization signal independent of other viral proteins (Coleman and Peeples, 1993), but what factors determine the nucleolar localization of NDV M protein and the effect of this localization on virus replication are not clearly understood.

Protein B23 (NPM1, nucleophosmin) is an abundant phosphoprotein localized mainly in the nucleoli that undergoes different phosphorylation events during the cell cycle (Okuwaki et al., 2002). It is a multifunctional protein that has nucleic acid binding, ribonuclease and molecular chaperone activities (Hingorani et al., 2000). Interestingly, although B23 is enriched in the nucleolus, it is demonstrated to be a nucleocytoplasmic transport protein (Fankhauser et al., 1991; Okuwaki et al., 2002). Owing to this ability, many of cellular proteins such as nucleolin (Li et al., 1996), p120 (Valdez et al., 1994) and ribosomal protein S9 (Lindstrom, 2012), and viral proteins such as Rex protein of human T-cell leukemia virus (Adachi et al., 1993), Rev protein (Fankhauser et al., 1991) and Tat protein of HIV-1 (Li, 1997), are reported to

* Corresponding author at: Yangzhou University, Animal Infectious Disease Laboratory, College of Veterinary Medicine, 12 East Wenhui Road, Yangzhou, Jiangsu 225009, China. Tel.: +86 514 8799 1416; fax: +86 514 8797 2591.
E-mail address: xfliu@yzu.edu.cn (X. Liu).

0042-6822/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2014.01.011

accumulate in the nucleolus by interaction with B23 via their nucleolar localization signals (NoLSs). Since B23 constantly shuttles between the nucleoli and the cytoplasm and numerous proteins to adopt nucleolar localization is correlated with interaction with B23, it has been proposed as a hub protein for nucleolar targeting proteins possessing a NoLS (Emmott et al., 2008; Lechertier et al., 2007; Sirri et al., 2008). Recent studies have also highlighted the fact that B23 is involved in additional nonclassical roles, including cell cycle regulation, cellular stress responses, and viral replication (Boisvert et al., 2007; Hiscox et al., 2010). Moreover, it is becoming more and more clear that different types of viruses targeting proteins to the nucleolus can induce important alternations of B23 and that these alternations participate in specific processes that are crucial for the outcome of virus infection, like viral DNA or RNA replication, virus assembly, and control of intracellular trafficking (Hiscox, 2002, 2007; Zakaryan and Stamminger, 2011).

We have hypothesized that NDV M protein might localize to the nucleolus by interaction with B23 and despoil the functions of B23 in the nucleolus to facilitate NDV replication. To this end, we first performed a yeast two-hybrid system and observed the interaction between M and B23 in yeast. This interaction was subsequently confirmed by GST pull-down assay and, more importantly, by co-immunoprecipitation assay in NDV-infected cells. Moreover, we demonstrated that NDV M protein accumulated in the nucleolus by binding the C-terminal amino acids 188–245 of B23 early in infection and resulted in the redistribution of B23 from nucleoli to the nucleoplasm later in infection. Further characterization revealed that knockdown of B23 or overexpression of B23 or M-binding B23-derived polypeptides remarkably reduced cytopathic effect (CPE) and decreased the replication efficiency of NDV. Therefore, the association of nucleolar protein B23 with NDV M protein may represent an important mechanism for NDV replication.

Results

The M protein interacts with B23 protein in yeast

Since the three most abundant nucleolar proteins – nucleolin, fibrillarin, and B23 – are able to interact with the viral proteins which localize to the nucleolus, we first performed the yeast two-hybrid system to confirm the interaction of M with them. In this assay, bait and prey plasmids co-transformed yeast colonies that grow on SD/-Trp/-Leu/-His/-Ade/X-α-Gal medium and turn blue are demonstrated to be positive. We found that only yeast colonies co-expressing BD-M/AD-B23 and BD-53/AD-T (positive control) grew on this medium and became blue (Fig. 1A, middle panel). In addition, β-galactosidase colony-lift filter assay simultaneously confirmed the interaction between M and B23 (Fig. 1A, right panel). We also carried out liquid β-galactosidase assays by co-transforming the recombinant plasmids to yeast cells. As shown in Fig. 1B, co-transformation of BD-M and AD-B23 resulted in a strong induction of β-galactosidase activity, significantly higher than that of the control and other co-transformations. These results collectively indicate that M and B23 interact specially with each other in yeast.

Interaction between M and B23 in vitro and in vivo

To verify and extend the binding data obtained in yeast two-hybrid assay, we performed GST pull-down experiments. A protein

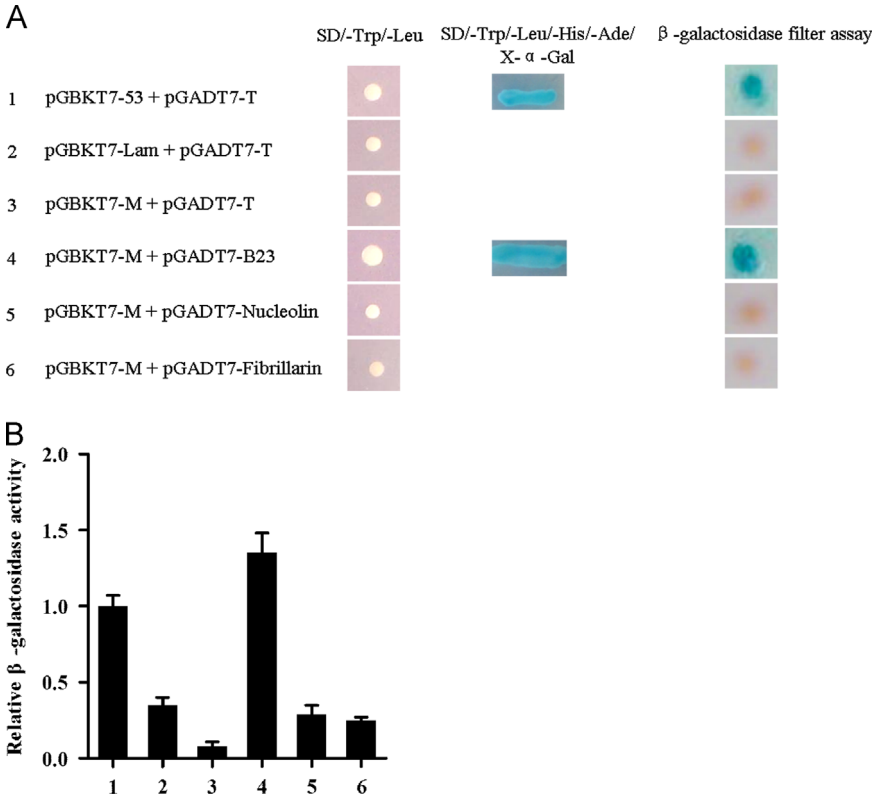


Fig. 1. The M protein interacts with B23 protein in yeast. (A) Yeast strain AH109 co-transformed with the indicated expression constructs were grown on SD/-Trp/-Leu/-His/-Ade/X-α-Gal medium. Yeast colonies growing on this medium and turning blue is the indication of interaction of the two expressed proteins. Examination of interaction of the two expressed proteins was also confirmed by β-galactosidase filter assay. The pGBKT7-53 and pGADT7-T or pGBKT7-Lam and pGADT7-T co-transformed group was used as positive control and negative control, respectively. (B) Quantification of β-galactosidase activity of the above combinations. Data shown are the means of three separate experiments and error bars are standard deviations.

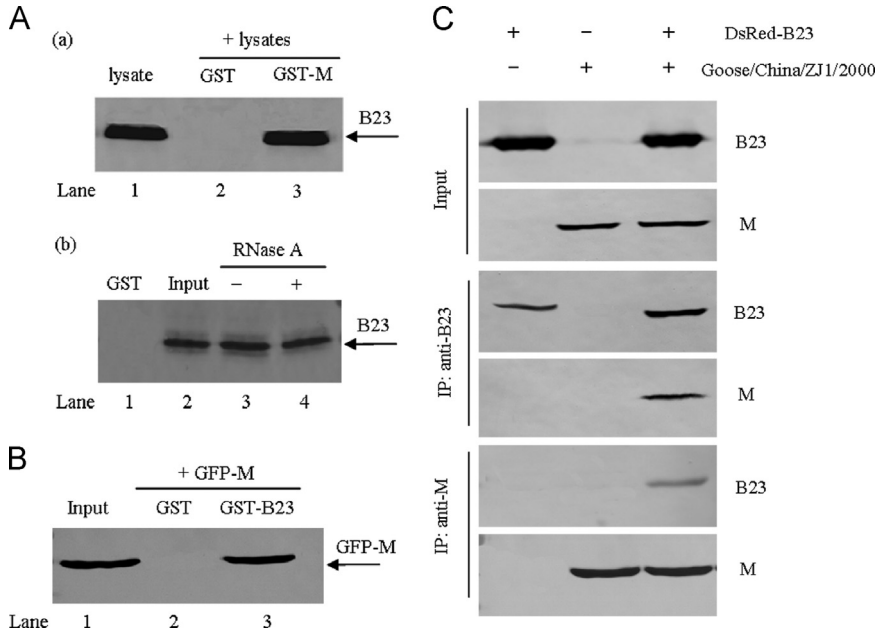


Fig. 2. Interaction between M and B23 *in vitro* and *in vivo*. (A) DF-1 cell lysates were incubated with glutathione-sepharose beads bound to either GST (lane 2) or GST-M fusion protein (lane 3). After washing, the bound proteins were separated by SDS-PAGE and transferred to a nitrocellulose membrane. Endogenous B23 was detected by Western blotting using an anti-B23 polyclonal antibody (upper panel). The untreated DF-1 cell lysates (lane 3) or DF-1 cell lysates treated with RNase A (lane 4) were incubated with GST-M, respectively. After washing, the bound protein was detected using an anti-B23 polyclonal antibody (lower panel). (B) M protein with GFP-tag was incubated with glutathione-sepharose beads bound to either GST (lane 2) or GST-B23 fusion protein (lane 3). After washing, the bound protein was detected using anti-M antibody. (C) Reciprocal co-immunoprecipitation assay of DsRed-B23 and M in DF-1 cells. DF-1 cells were transfected with plasmid encoding a DsRed-tagged B23 protein and were infected with NDV at an MOI of 0.1. Cells were lysed at 24 hpi, and a co-immunoprecipitation assay was performed using either anti-B23 (middle panel) or anti-M (lower panel) antibodies. Immunoprecipitated proteins were detected by Western blotting using anti-M or anti-B23 antibodies. The input samples were shown in upper panel.

binding assay of GST-M fusion protein to endogenous B23 protein showed that endogenous B23 was pulled-down by GST-M protein but not by GST (Fig. 2A-a). In turn, exogenous GFP-M protein could bind to GST-B23 protein, but not GST (Fig. 2B). Since B23 could interact with protein and form a stable complex through RNA (Huang et al., 2005; Pinol-Roma, 1999), we examined whether this is the case in M-B23 interaction. The lysates of DF-1 cells were treated with RNase A prior to GST pull-down assay, but no significant differences were seen before and after RNase A treatment (Fig. 2A-b). Together, these results demonstrate that that M protein interacts with B23 protein *in vitro*.

To further confirm the interaction between M and B23 in avian cells, we performed co-immunoprecipitation experiments with DF-1 cells transiently transfected with plasmid encoding DsRed-tagged B23 protein (DsRed-B23) and infected with NDV. Expression of the recombinant DsRed-B23 and viral M proteins was detected with anti-B23 and anti-M antibodies, respectively (Fig. 2C, upper panel). Cell lysates were then immunoprecipitated with anti-B23 antibody and subsequently immunoblotted with anti-M antibody. As shown in the middle panel of Fig. 2C, co-immunoprecipitation of M with B23 was detected, confirming their physical interaction in virus-infected cells. The specificity of the M-B23 interaction was further demonstrated by detection of DsRed-B23 protein in reciprocal co-immunoprecipitation assays using anti-M antibody (Fig. 2C, lower panel). These results clearly demonstrate that M protein physically interacts with B23 *in vivo*.

Intracellular distribution of M and B23

The interaction between M and B23 indicated that the two proteins may have the similar intracellular localization. To investigate the subcellular localization of M and B23, we first performed colocalization studies with HEK-293T cells transiently transfected with plasmids encoding GFP-tagged M and DsRed-tagged B23 proteins. Results showed that M protein either fused to the N-terminus or C-terminus of GFP exhibited the clear colocalization with B23 in the nucleolus (Fig. 3A). Detection of the intracellular localization of GFP-M and DsRed-B23 proteins by Western blot confirmed the distribution of the two fusion proteins had the same distributions as cellular marker proteins (nucleolin for the nucleolus and nucleoplasmin 3 (NPM3) for the nucleoplasm) (Fig. 3B).

To validate whether this intracellular localization occurs in virus-infected cells, DF-1 cells infected with NDV were fixed at different time points and the intracellular localization of M and B23 was examined by indirect immunofluorescent assay. At 5 hpi, the M protein was concentrated in the nucleus, particularly in the nucleolus, showing the same intracellular localization with B23 in the nucleolus (Fig. 3C). At 8 hpi, the M protein was concentrated more in the nucleolus and B23 protein was redistributed in the nucleoplasm and the nucleus in addition to the nucleolus. But at 12 hpi, the M protein was distributed in the whole cells and B23 protein was localized mainly in the nucleoplasm and the nucleus (Fig. 3C). Immunoblot analysis also confirmed the intracellular localization of the two proteins (tubulin for the cytoplasm, lamin B1 for the nucleus, NPM3 for the nucleoplasm and nucleolin for the nucleolus) (Fig. 3D). All together, these results demonstrate that B23 co-localizes with M in the nucleolus early in infection, but it can be redistributed to the nucleoplasm later in infection.

Mapping binding domains between M and B23

To determine the regions of M and B23 that are responsible for interaction with each other, a series of M and B23 deletion mutants were constructed to seek their binding domains utilizing His and GST pull-down assays (Fig. 4A and B, upper panels). Bacterially purified 6His-tagged full-length M and its deletion mutants were first detected by Western blot analysis (Fig. 4A, Input). Bands of these proteins quantitated by densitometry with the ImageJ 1.2.4 program revealed that 6His-M and its deletion mutants were expressed at levels that were similar to that of 6His

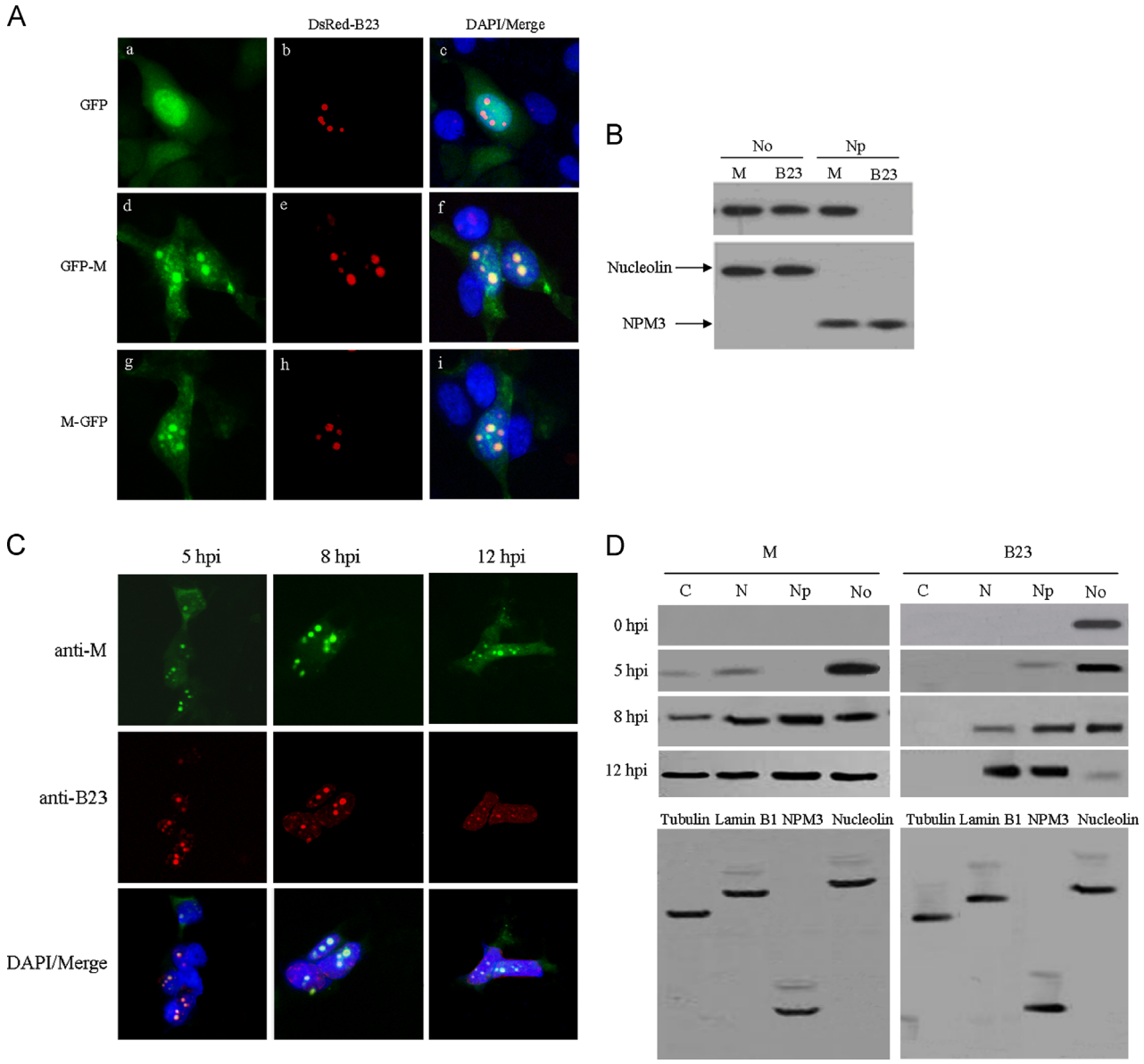


Fig. 3. Intracellular distribution of M and B23. (A) HEK-293T cells were transiently transfected with the plasmids expressing GFP-tagged M and DsRed-tagged B23. After 24 h transfection, subcellular localization of GFP-M or M-GFP and DsRed-B23 was observed under fluorescent microscope. DAPI was used to stain nuclei. Original magnification was 1 × 200. (B) Western blotting analysis of the nucleolar (lane No) and nucleoplasmic (lane Np) fractions extracted form HEK-293T cells using anti-M and anti-B23 antibodies. Nucleolin for the nucleolus and nucleoplasmin 3 (NPM3) for the nucleoplasm were used as cellular markers. (C) DF-1 cells were infected with NDV at an MOI of 0.1 and fixed at different times, followed by immunofluorescent staining. DAPI was used to stain nuclei. Original magnification was 1 × 200. (D) Western blot analysis of the cytoplasmic (lane C), nuclear (lane N), nucleoplasmic (lane Np) and nucleolar (lane No) subfractions in NDV-infected DF-1 cells presented in (C). Tubulin for the cytoplasm, Lamin B1 for the nucleus, NPM3 for the nucleoplasm and Nucleolin for the nucleolus were used as cellular markers.

tag alone (Fig. S1). Binding studies showed that the N-terminus (aa 30–60) of M was essential for B23 binding, since 6His-M (60–364) and 6His-M (Δ30–60) lost their binding activity to B23 (Fig. 4A, lower panel). As negative control, GST-B23 did not bind to 6His tag alone.

Various functional domains have been identified within B23 protein (Hingorani et al., 2000), including N-terminal oligomerization domain (OligoD) bearing chaperone activity, the C-terminal nucleic acid binding domain (NBD), and two central acids domains for histone binding (HistonD). To examine which domain of B23 interacts with M, similar GST pull-down approach was performed. Bacterially purified GST-tagged full-length B23 and its deletion mutants were also detected and analyzed as described in Fig. 4A. Results showed that these fusion proteins were normally expressed and had the same expression levels as GST alone (Fig. 4B, Input, and Fig. S1). The pull-down data revealed that only GST-B23 including residues 188–245 could be pulled down by 6His-M (Fig. 4B, lower panel), indicating that the C-terminal nucleic acid-binding domain of B23 was essential for its interaction with M.

B23 depletion leads to M accumulation in the nucleus

The facts that B23 transports nucleolar targeting proteins to the nucleolus and M interacts with B23 suggest that M might accumulate in the nucleolus by binding B23. To test this hypothesis, the subcellular localization of M in B23-depleted DF-1 cells was investigated. Three pairs of synthesized B23 siRNAs were transfected into DF-1 cells respectively, and one (B23 RNAi#2) could effectively lower the expression level of B23 without causing discernable changes in cell morphology (Fig. 5A and B). In addition, we examined the viability of those cells receiving siRNA

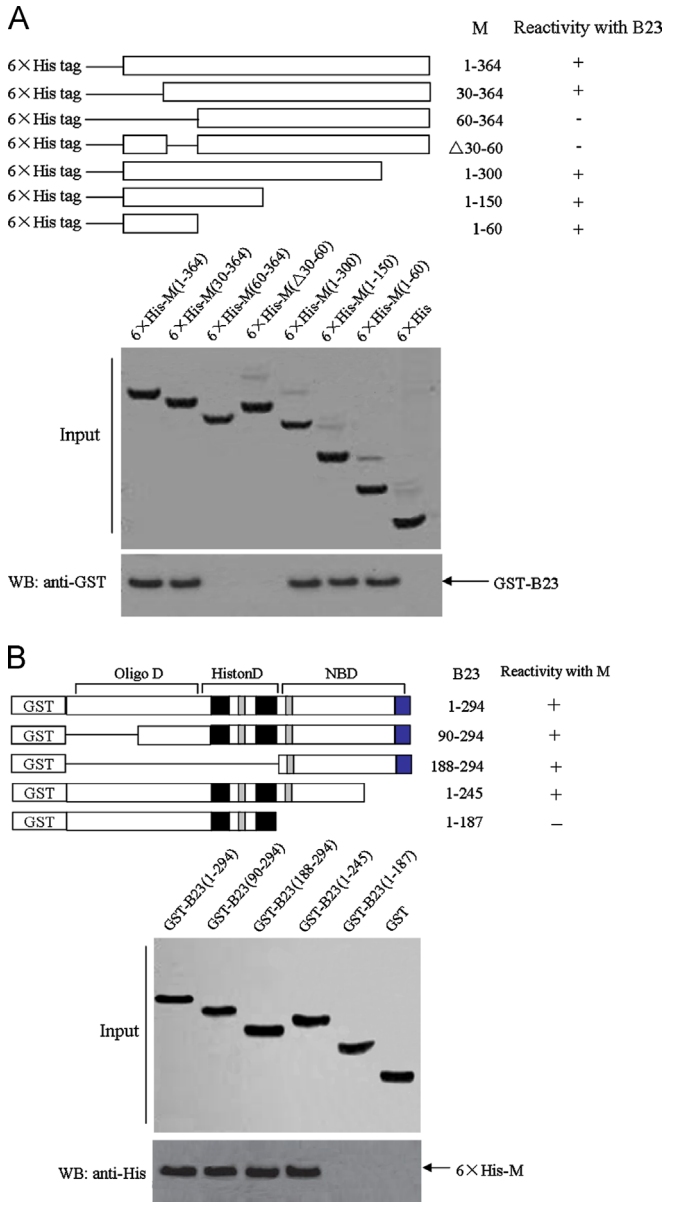


Fig. 4. Mapping binding domains between M and B23. (A) His pull-down mapping domains of M for B23 binding. Upper panel, schematic representation of full-length and deletion mutants of 6 × His-tagged M (6His-M). Lower panel, various purified 6His-M or its deletion mutants as indicated were incubated with GST-B23 protein and the pulled down proteins was immunoblotted against GST. (B) GST pull-down probing regions of B23 for M binding. Schematic diagram of full-length and deletion mutants of GST-tagged B23 (GST-B23) is shown on the upper panel. GST pull-down assays were performed through incubation of various purified GST-B23 or its deletion mutants with 6His-M protein. Pull-down fractions were detected by immunoblotting against His (lower panel).

using trypan blue exclusion and MTT assays. Results showed that there was no difference between B23 RNAi and RNAi control in terms of viability of transfected cells (data not shown). We then infected DF-1 cells receiving this siRNA or control siRNA with the NDV strain Goose/China/ZJ1/2000. As a result, at 8 hpi, B23 depletion caused M mainly in the nucleus and markedly reduced the nucleolar accumulation of M compared to that of RNAi control (Fig. 5C). Similar results were obtained by examining the intracellular distribution of M and B23 in control siRNA- or B23 siRNA-treated DF-1 cells through immunoblot analysis (Fig. 5D). Together with the above results, these data reveal that M accumulates in the nucleolus by binding B23.

Knockdown of B23 inhibits NDV replication in DF-1 cells

Since B23 plays an essential role in the nucleolar localization of M, this might be critical for the replication of NDV. To this end, siRNA-mediated knockdown of B23 in DF-1 cells infected with NDV was investigated. As shown in Fig. 6A C, knockdown of B23 markedly reduced NDV-induced CPE at 24 hpi, accompanied by significant reduction of viral loads in the cell culture supernatants and cell pellets in comparison to that of RNAi control ($P < 0.01$) (Fig. 6D and E). Consistently, at 24 hpi, the HA titers in the culture supernatants and cell pellets of B23 siRNA-treated cells dramatically decreased when compared to that of normal cells (Fig. 6F and G), although the difference of HA titers in the cell pellets between RNAi control and B23-RNAi was not significant (Fig. 6G). To further determine the effect of B23 knockdown on NDV replication, the mRNA expression levels of NDV M gene in NDV-infected B23-RNAi cells were examined. We found that the mRNA expression level of NDV M gene in B23-RNAi cells significantly decreased in comparison to that of RNAi control and normal cells either in the culture supernatants or in the cell pellets ($P < 0.001$) (Fig. 6H and I), indicating that NDV growth was arrested in cells with a reduced level of B23. Together, these data demonstrate that B23 plays a critical role in NDV replication.

Overexpression of B23 or M-binding B23 fragments restricts NDV replication

Now that knockdown of B23 significantly affected NDV replication in DF-1 cells, it was intriguing to determine whether overexpression of B23 or M-binding B23 fragments could also affect NDV replication. For this purpose, DF-1 cells overexpressing DsRed, DsRed-B23 (1–294), DsRed-B23 (Δ188–245) or DsRed-B23 (188–245) were generated. The effects of these overexpressed proteins on the intracellular localization of NDV M protein were first examined. We found that overexpression of DsRed-B23 (1–294) or DsRed-B23 (188–245) restricted M protein in the nucleolus or in the cytoplasm, while overexpression of DsRed or DsRed-B23 (Δ188–245) did not affect the intracellular localization of M protein (Fig. 7A). These results strongly suggest that the amino acids 188–245 of B23 are critical for M binding and that binding to the B23 present in the cytoplasm is necessary for M nucleolar localization.

Then the effects of B23 or M-binding B23 fragments overexpression on NDV replication were investigated. Results showed that overexpression of DsRed-B23 (1–294) or DsRed-B23 (188–245) markedly mitigated NDV-induced CPE in DF-1 cells, whereas overexpression of DsRed or DsRed-B23 (Δ188–245) had significant CPE, which was similar to normal cells infected with NDV (Fig. 7B). In addition, the HA titers in DF-1 cells overexpressing DsRed-B23 (1–294) or DsRed-B23 (188–245) dramatically decreased when compared to that of normal cells ($P < 0.01$) (Fig. 7C and D). Moreover, overexpression of DsRed-B23 (1–294) or DsRed-B23 (188–245) significantly inhibited the mRNA expression levels of NDV M gene in the cell culture supernatants and in the cell pellets ($P < 0.01$) (Fig. 7E and F), suggesting that NDV growth was reduced with overexpression of B23 or M-binding B23 fragments. These data undoubtedly consolidate the results that NDV replication is associated with B23.

Discussion

The nucleolus is the most distinct structure in the cell nucleus, but the nucleolar structure are constantly changing due to the effect of metabolic conditions, and virus infection represents one of the major challenges to nucleolar function (Greco, 2009; Hiscox, 2002; Hiscox et al., 2010). Within recent years, increasing evidence

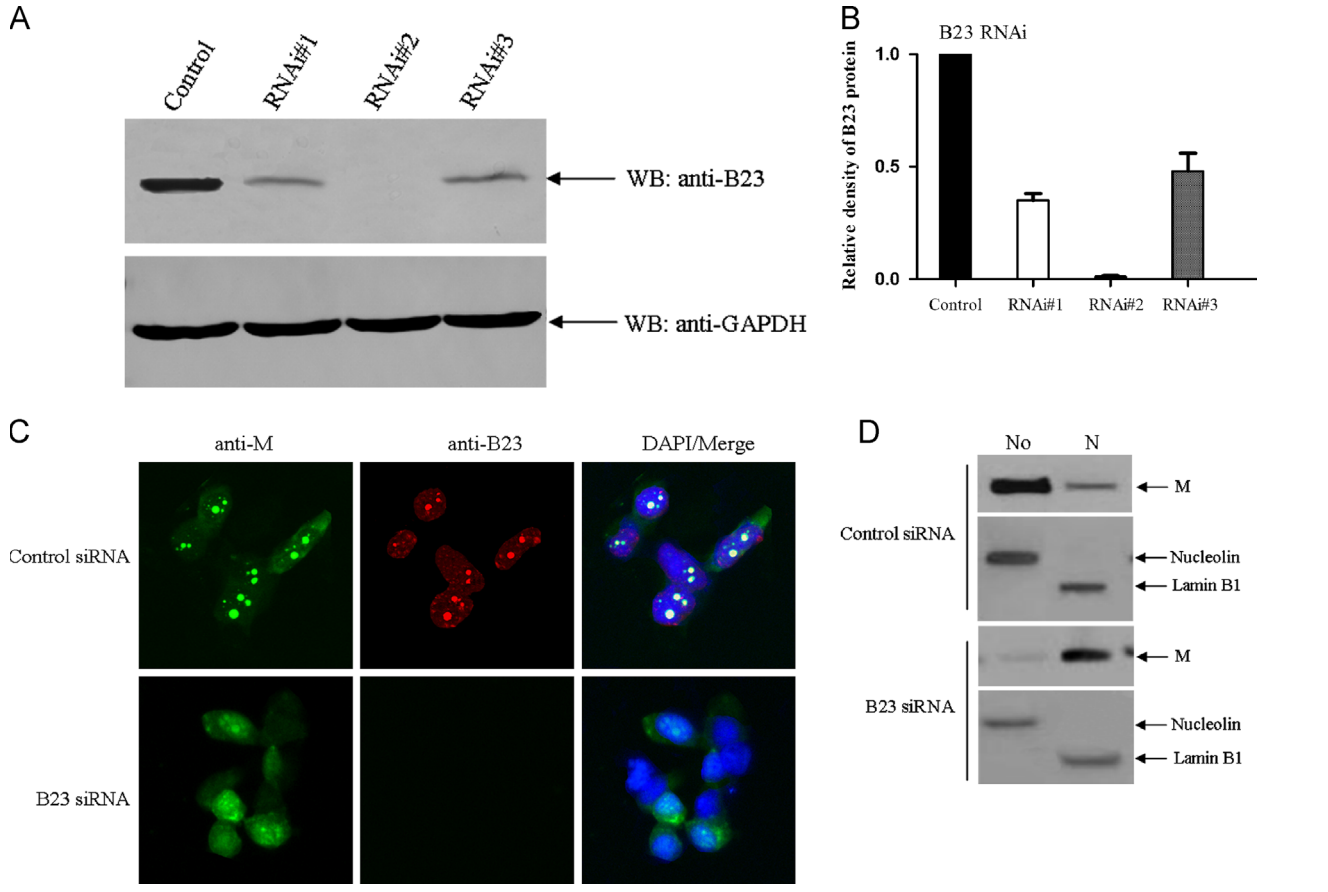


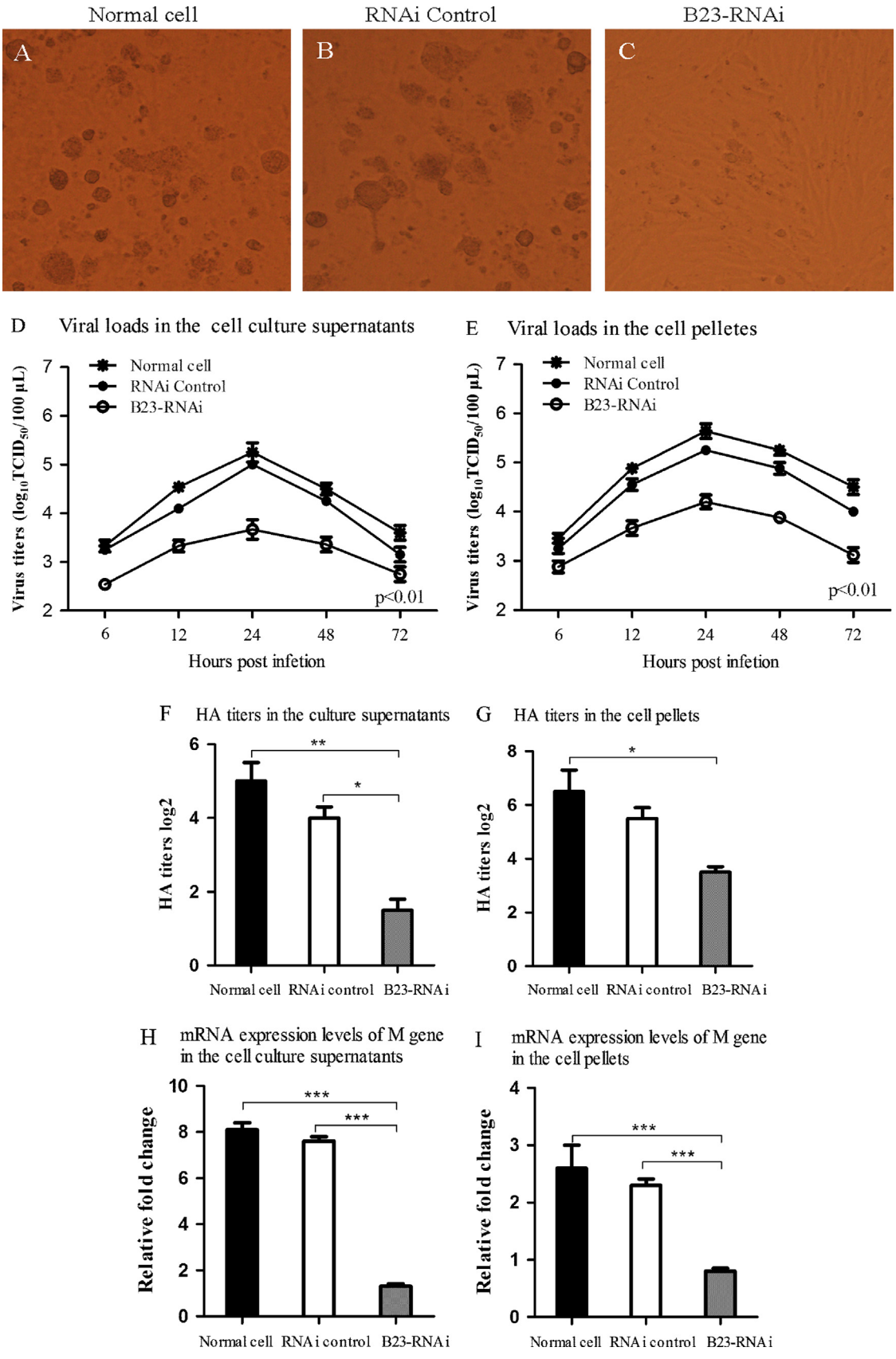
Fig. 5. B23 depletion leads to M accumulation in nuclei. (A) Effects of B23 RNAi on the expression of endogenous B23. DF-1 cells were transfected with siRNA (#1–3) or controls. After 48 h transfection, cell lysates were prepared and examined by Western blot with anti-B23 antibody. Endogenous GAPDH expressions were used as internal controls. (B) The relative levels of B23 in B23 siRNA-treated cells. The density of bands in (A) was quantitated by densitometry. The relative levels of B23 were calculated from three independent experiments performed as follows: the band density of B23/the band density of GAPDH, with standard deviations indicated by error bars. (C) DF-1 cells were transfected with control siRNA or B23 siRNA. Forty-eight hours after transfection, cells were infected with NDV at an MOI of 1. At 8 hpi, subcellular localization of M and B23 in infected cells were visualized by staining with anti-M and anti-B23 antibodies followed by secondary antibody conjugated with fluorescent probes. DAPI was used to stain nuclei. Original magnification was 1 × 200. (D) Immunoblot analysis of intracellular distribution of M and B23 in control siRNA- or B23 siRNA-treated DF-1 cells. No represents the nucleolus and N represents the nucleus. Cellular markers are presented.

has revealed that distinct types of viruses require the nucleus and in particular the nucleolus to target proteins indispensable for their replication and pathogenic process (Hiscox et al., 2010; Huang et al., 2001; Sirri et al., 2008). For paramyxoviruses, the nucleolar proteins reported to interact with viral proteins are the association of B23 with human respiratory syncytial virus (HRSV) M protein (Oliveira et al., 2013). Although the M protein of HRSV is localized predominantly in the nucleus early in infection (Ghildyal et al., 2003), the interaction of B23 with M protein indicates that B23 may be recruited by HRSV M protein to assist its assembly process, though this hypothesis has not been demonstrated. Unlike the intracellular localization of HRSV M protein, NDV M protein is detected in the nucleus and the nucleolus early in infection and remains concentrated in the nucleolus throughout infection (Duan et al., 2013a, 2013b; Peeples et al., 1992), indicating a different role of NDV M protein in the nucleolus for NDV replication. However, relatively little is known about the contribution of M-interacting nucleolar proteins to NDV replication.

In the present study, nucleolar protein B23 was identified as a NDV M-interacting factor, and B23-binding ability was mapped to a region of M protein located at amino acids 30–60. In addition, siRNA-mediated depletion of B23 resulted in the accumulation of M in the nucleus, demonstrating that NDV M accumulated in the nucleolus by interaction with B23. B23 as a hub protein targeting cellular or viral proteins to the nucleolus has been described previously (Li, 1997; Lindstrom, 2012; Valdez et al., 1994; Wang et al., 2011). It has been proven that these proteins are transported to the nucleolus by binding B23 via intrinsic NoLS (Emmott and Hiscox, 2009; Hiscox, 2002). Although several NoLSs have been described, no obvious consensus sequence has been identified so far due to the diversity of NoLSs. Nevertheless, majorities of NoLSs responsible for protein nucleolar localization are rich in basic residues (Hiscox, 2002). The identified amino acid residues 30–60 (^{30}TGDG**KK**QITPQY**R**IQ**R**LDSWTDS**K**EDSVFIT^{60}) of M contained basic residues and deletion of this fragment lost its binding activity to B23. Previous finding showed that deleting residues 43–101 of M abrogates the localization of M in the nucleolus (Coleman and Peeples, 1993). These results suggest that that the residues aa 30–60 may be the NoLS of M. However, much more work is needed to confirm this speculation. Recently, several studies have shown that mutation of the NoLS in viral proteins in the context of recombinant viruses attenuates virus replication and pathogenicity (Lee et al., 2006; Mori et al., 2005). The candidate NoLS in NDV M protein provided us the chance to further investigate the biological significance of the nucleolar localization of M protein in NDV replication cycle.

In addition, binding studies confirmed that the residues 188–245 of B23 that belongs to the C-terminal nucleic acid-binding domain were found to be required for interaction with NDV M protein, which is similar to the binding domain of B23 to protein p120 (Valdez et al., 1994), nucleolin (Li et al., 1996), and HIV-1 Tat (Li, 1997). The intracellular localization of M and B23 (188–245)

showed that M co-localized with B23 (188–245) in the cytoplasm in NDV-infected DF-1 cells overexpressing DsRed-B23 (188–245), suggesting that the binding of M to the residues 188–245 of B23 in the cytoplasm was essential for the nucleolar localization of M. This is consistent with the previous finding observed in the interaction between HIV-1 Tat and B23 (187–255) (Li, 1997). B23



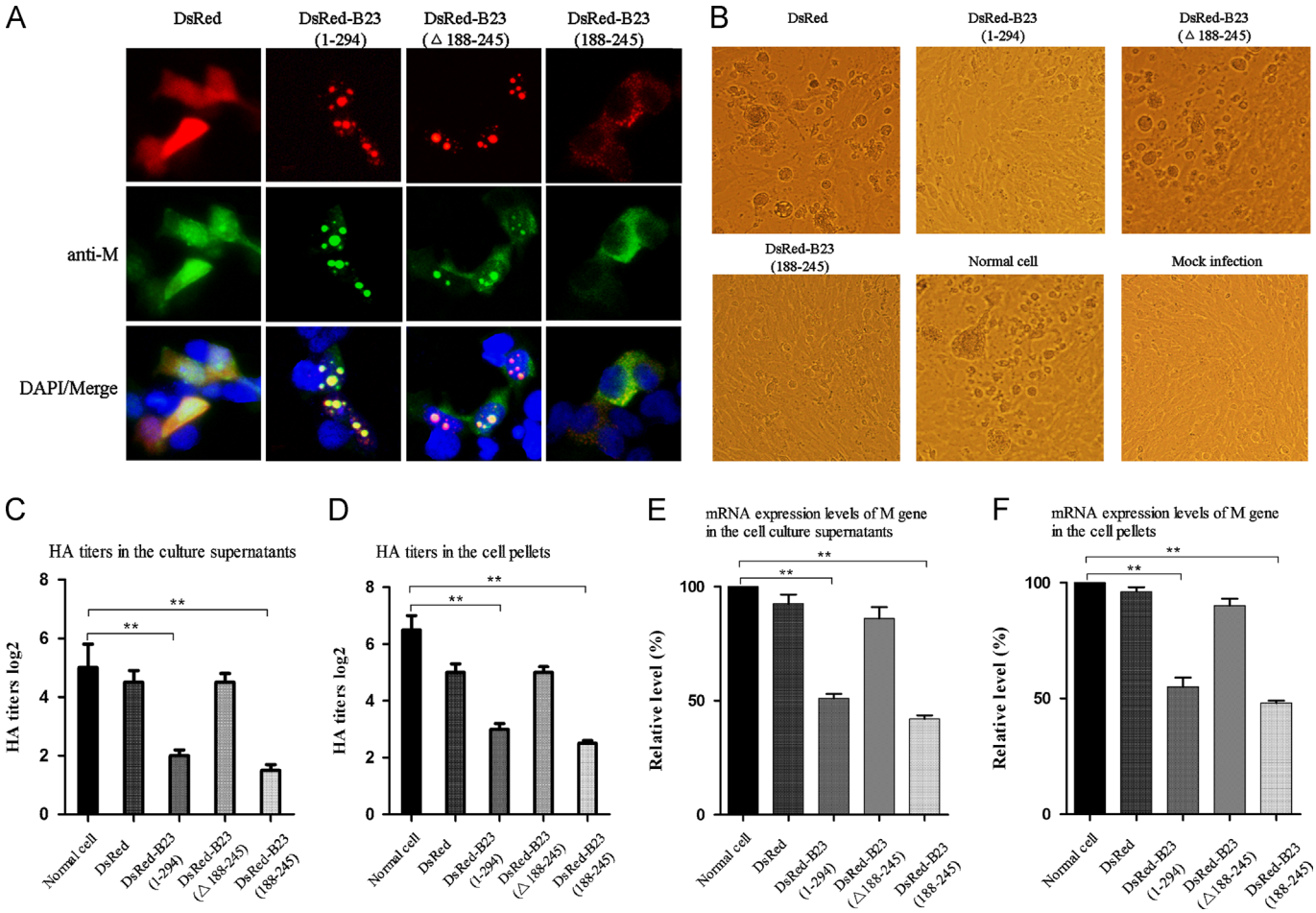


Fig. 7. B23 or M-binding B23 fragments overexpression restricts NDV replication. (A) The intracellular localization of M and B23 or its mutants. DF-1 cells overexpressed DsRed, DsRed-B23 (1-294), DsRed-B23 (Δ188–245) or DsRed-B23 (188–245) were infected NDV at an MOI of 1. At 12 hpi, cells were fixed and visualized by staining with anti-M antibody followed by secondary antibody conjugated with fluorescent probes. DAPI was used to stain nuclei. Original magnification was 1 × 200. (B) Morphological changes of DF-1 cells during NDV infection. DF-1 cells overexpressed the indicated proteins were infected with NDV at an MOI of 1. At 24 hpi, cells were examined by phase-contrast microscopy. Original magnification was 1 × 100. (C and D) Quantitative analysis of NDV HA titers as examined in B at 24 hpi. The graphs show the average of HA titers from three independent experiments. (E and F) DF-1 cells were treated as described in (B). At 24 hpi, the culture supernatants (E) and cell pellets (F) were collected for detecting mRNA expression of NDV M gene using a real-time RT-PCR. The mRNA levels of NDV M gene in the cell pellets were normalized to housekeeping gene GAPDH, and each mRNA expression of NDV M gene was calculated relatively to that of normal cell group (× 100%). Results are representative of three independent experiments. The significance of the difference was performed by the Independent-Samples *T*-test (***$p < 0.001$, **$p < 0.01$ and *$P < 0.05$).

is a multifunctional protein that has nucleic acid-binding, ribonuclease and molecular chaperone activities (Huang et al., 2005). Studies have demonstrated that the C-terminal nuclei acid-binding domain of B23 is involved with recognition at various levels, such as its association with preribosomal particles through RNA binding in the nucleolus, targeting proteins bound to B23 to specific sites during the ribosome assembly process, and acting as substrate recognition for the ribonuclease activity of B23 (Hingorani et al., 2000; Huang et al., 2005; Itahana et al., 2003). Previous study has speculated that NDV M protein localizes to the nucleus and the nucleolus to inhibit host ribosome assembly and protein synthesis (Peeples et al., 1992). The association of the C-terminal nuclei acid-binding domain of B23 with M protein gave us one possible explanation that M's ability to achieve this inhibition process may be associated with the disruption of structure and function of B23 caused by M in the nucleolus.

Several other studies have shown that B23 redistributed to other cellular localizations during virus infection is important for the virus life cycle. For example, Japanese encephalitis virus core protein translocates B23 from nucleoli to the cytoplasm and facilitates virus replication (Tsuda et al., 2006). Herpes simplex virus 1 (HSV-1) UL24 protein causes the dispersal of B23 to the cytoplasm, and the involvement of UL24 in the dispersal of B23 promotes the nuclear egress of viral particles (Lymberopoulos et al., 2011). More recently, studies have demonstrated that adenoviral protein V promotes the B23 translocation from the nucleoli to the nucleoplasm, and this translocation is correlated with adenoviral replication (Matthews, 2001; Ugai et al., 2012).

Fig. 6. Knockdown of B23 inhibits NDV replication in DF-1 cells. (A, B, and C) Morphological changes of DF-1 cells after NDV infection. Normal cells (A), RNAi control cells (B), and B23-RNAi cells (C) were infected with NDV at an MOI of 1. Twenty-four hour after NDV infection, cells were examined by phase-contrast microscopy. Original magnification was 1 × 100. (D and E) Knockdown of B23 reduced NDV growth. Normal cells, RNAi control cells and B23-RNAi cells were infected with NDV at an MOI of 1. At different time points (6, 12, 24, 48 and 72 hpi), the viral loads in the cell culture supernatants (D) and cell pellets (E) were determined by $TCID_{50}$ in DF-1 cells. The graphs show the average of viral titers in DF-1 cells from three independent experiments. (F and G) Quantitative analysis of NDV HA titers as examined in (D) and (E) at 24 hpi. The graphs show the average of HA titers from three independent experiments. (H and I) Normal cells, RNAi control cells and B23-RNAi cells were infected with NDV at an MOI of 1. Twenty-four hours after NDV infection, the culture supernatants (H) and cell pellets (I) were examined by a real-time RT-PCR to determine the mRNA expression levels of NDV M gene. The significance of the difference between B23-RNAi and RNAi control treatments was determined using the Independent-Samples *T*-test (***$p < 0.001$, **$p < 0.01$ and *$P < 0.05$). The graphs show the average of mRNA levels of NDV M gene in DF-1 cells from three independent experiments.

Although the redistributions of B23 caused by different viruses have the difference, depletion or overexpression of B23 reduces virus replication. In NDV-infected DF-1 cells, we also found that B23 co-localized with M in the nucleolus early in infection, but it was redistributed to the nucleoplasm later in infection. The critical role of B23 in NDV replication was subsequently confirmed by the fact that B23 knockdown or overexpression affected the normal intracellular localization of M protein and markedly reduced CPE and inhibited NDV replication, demonstrating the equally important role of B23 in NDV replication.

In summary, we demonstrated that B23 facilitates NDV replication by transferring M to the nucleolus through M-B23 interaction. We hypothesized that NDV replication was enhanced by disrupting the structure and function of B23 to inhibit host ribosome biogenesis and protein synthesis. Although our studies demonstrated the importance of M-B23 interaction for NDV replication, the precise role of B23 in NDV pathogenesis remains poorly understood. Further investigations capitalizing on proteomics, viral genetics and cellular imaging techniques will be necessary to shed light on the all aspects of correlations between B23 and NDV infection.

Materials and methods

Plasmids and antibodies

To generate GFP, GST or His epitope-tagged full-length protein of M, PCR was performed to amplify the open reading frame (ORF) of NDV M gene from the full-length cNDA clone of NDV strain Goose/China/ZJ1/2000 (Liu et al., 2007). The PCR product was digested with *Xho*I-*Bam*H I and then inserted into pEGFP-C1, pGEX6p-1 and pET-His (Clontech). Truncation mutants of M were constructed by inserting PCR-generated fragments into the pET-His vector. Plasmid pGBKT7-M was generated by inserting M ORF in frame with GAL4 DNA binding domain into pGBKT7 (Clontech). The B23 ORF or its deletion mutants was amplified by PCR using pDsRed-B23.1 (Duan et al., 2013a, 2013b) as the template and then inserted into the indicated vectors. The chicken nucleolin and fibrillarin ORF were amplified from cDNA of DF-1 cells by PCR using the specific primers. Plasmids pGADT7-Nucleolin and pGADT7-Fibrillarin were generated by inserting the nucleolin and fibrillarin ORF in frame into the pGADT7 at the same restriction sites. All the recombinant plasmids were confirmed by PCR, restriction digestion and DNA sequencing.

Primary antibodies rabbit anti-B23 polyclonal antibody (sc-5564), mouse anti-GST monoclonal antibody (sc-374171) and mouse anti-His monoclonal antibody (sc-8036) were purchased from Santa Cruz Biotechnology (USA). Rabbit anti-Tubulin polyclonal antibody (ab125267), rabbit anti-Lamin B1 polyclonal antibody (ab16048), rabbit anti-NPM3 polyclonal antibody (ab103779) and rabbit anti-Nucleolin polyclonal antibody (ab70493) were purchased from Abcam (UK). Rabbit anti-NDV M polyclonal antibody was kindly provided by Dr. Chan Ding (Shanghai Veterinary Research Institute, China).

Cell culture, transfection and fluorescence microscopy

The chicken embryo fibroblast cell line DF-1 and Human embryonic kidney (293T) were grown and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) in a humidified 5% CO_2 incubator at 37 °C. For the transfection experiments, 5×10^4 HEK-293T cells were grown on glass coverslips in 12-well plates with approximately 60% confluence. Transient transfection of all plasmids was performed with FuGENE® HD Transfection Reagent (Roche). Twenty-four hours after transfection, cells expressing fluorescence-fused proteins were fixed with 4% paraformaldehyde for 20 min, permeabilized with 0.2% Triton X-100 for 5 min, and then observed under a Leica fluorescence microscope (Germany). The transfected cells were all counterstained with DAPI (Sigma) to detect nuclei.

Yeast two-hybrid assays

In yeast two-hybrid assay, pGBKT7-M was used as bait and pGADT7-B23, pGADT7-Nucleolin and pGADT7-Fibrillarin were used as prey. The plasmids expressing bait protein and prey protein were co-transformed into the yeast strain AH109 according to yeast protocols handbook. Yeast colonies were plated onto SD/-Trp/-Leu medium to grow and then transferred onto the plates SD/-Trp/-Leu/-His/-Ade containing X-α-Gal to check for expression of the MEL1 reporter gene. Only yeast colonies that grow on this medium and turn blue were evaluated as positive. Additionally, yeast colonies plated on SD/-Trp/-Leu medium were transferred into nitrocellulose filters and assayed for β-galactosidase activity as previously described (Eulalio et al., 2006). For quantitative β-galactosidase assays, the procedures for yeast cell transformation, screening for growth in the absence of histidine and measurement of β-galactosidase activity followed the yeast protocols handbook (Clontech).

GST pull-down assays

The interaction between M and B23 *in vitro* was examined by GST pull-down assays. The GST-M and GST-B23 recombinant proteins were purified from bacteria transformed with pGEX6p-M and pGEX6p-B23 vectors, respectively. DF-1 cells lysed in ice-cold lysis buffer (Beyotime, China) were treated or untreated with RNase A, and 1 mg of cell extracts were incubated for 3 h at 4 °C with 10 μg of GST-M or GST together with glutathione-Sepharose. The beads were extensively washed in lysis buffer, size-fractionated by SDS-PAGE, and immunoblotted for B23 using anti-B23 antibody. In turn, GST pull-down assay using GST-B23 protein and exogenous GFP-M protein derived from pEGFP-M transfected HEK-293T cells was performed as described above.

Co-immunoprecipitation and western blotting

DF-1 cells grown in 35 mm dishes were transfected with pDsRed-B23 plasmid for 24 h and then infected with NDV strain Goose/China/ZJ1/2000 at a multiplicity of infection (MOI) of 0.1. At 24 h post-infection (hpi), cells were washed and lysed with immunoprecipitation buffer (Invitrogen). After centrifugation, the supernatant was incubated with anti-B23 or anti-M antibody for 2 h. The immune complexes were recovered by adsorption to protein A+G-Sepharose (Sigma) overnight at 4 °C. After five washes in immunoprecipitation buffer, the immunoprecipitates were analyzed by Western blot analysis.

Immunofluorescence analysis of virus-infected cells

DF-1 cells were infected with NDV at an MOI of 0.1 and prepared for immunofluorescence analysis at 5 hpi, 8 hpi and 12 hpi, respectively. At the indicated times, cells were washed with PBS and fixed with 4% paraformaldehyde for 20 min, washed three times with PBS, and then permeabilized with 0.2% Triton X-100 in PBS for 5 min. Cells were rinsed with PBS and blocked with 10% FCS in PBS for 30 min, and then incubated with anti-B23 and anti-M antibodies diluted in PBS containing 10% FCS for 1 h. After three washes with PBS, the cells were incubated with Alexa Fluor 488 goat anti-mouse immunoglobulin G and Alexa Fluor 594 goat anti-rabbit immunoglobulin G antibodies (Invitrogen) for 1 h.

Cells were also counterstained with DAPI to detect nuclei, and images were captured with a Leica fluorescence microscope (Germany). Images were processed with Adobe Photoshop.

siRNA treatment and virus infection

The sequences of siRNAs designed to knockdown B23 in DF-1 cells were: RNAi#1 (sense, 5′-CAGUUUCACUAGGUGGAUUUGAGAU-3′; antisense, 5′- AUCUCAAAUCCACCUAGUGAAACUG-3′), RNAi#2 (5′-GAGCCAAAGACGAAUUACAUGUUGU-3′; antisense, 5′- ACAA-CAUGUAAUUCGUCUUUGGCUC-3′), RNAi#3 (5′-CACCACCAUUUGU-CUUGAGGUUAAA-3′; antisense, 5′-UUUAACCUCAAGACAAAUGG-UGGUG-3′). Negative siRNA control (Cat. no. 12935-400) and transfection reagents were purchased from Invitrogen. For transfection with the interference RNAs against B23, low-passage DF-1cells were transfected with siRNA at a confluence of 80% on 35 mm dishes, and the knockdown efficiency was checked by immunoblot analysis after 48 h transfection. Nucleoli were isolated from DF-1 cells as previously described (Andersen et al., 2005), with detailed protocol at http://www.lamondlab.com/f7nucleolarprotocol.htm. To study the effect of B23 knockdown on the replication of NDV, Goose/China/ZJ1/2000 was used to infect B23 siRNA-treated DF-1 cells at an MOI of 1. The cell culture supernatants and cell pellets were collected at different time points (6, 12, 24, 48, 72 hpi), and the virus titers were determined as 50% tissue culture infective dose ($TCID_{50}$) in DF-1 cells. HA titers of NDV were performed as previously described (Li et al., 2013).

B23 or its mutants overexpression and virus infection

To study the effect of B23 or M-binding B23 fragments over-expression on the replication of NDV, the constructed plasmids or empty vector controls were transfected into DF-1 cells to over-express DsRed-B23 (1–294), DsRed-B23 (Δ188–245) or DsRed-B23 (188–245) or DsRed. DF-1 cells were then infected with NDV at an MOI of 1 and the HA titers and mRNA expression levels of NDV M gene in the culture supernatants and cell pellets were examined at 24 hpi, respectively. The relative expression levels of NDV M gene in cells expressing B23 or its mutants were calculated in relation to that of normal cells after NDV infection.

Real-time quantitative PCR analysis of M gene mRNA

For quantification of NDV M gene mRNA, a SYBR green-based real-time PCR method (TaKaRa) was used, and GAPDH mRNA was quantified to normalize the total RNA concentration between different samples. The primers for M gene detection were M-F (5′-GCTTGTAAGGCGAGAGGTG-3′) and M-R (5′-AACCTGGGGA-GAGGCATTTG-3′), and those for GAPDH were G-F (5′-ATCACAGC-CACACAGAAGACGG-3′) and G-R (5′-CTTTCCCCACAGCCTTAGCAGC-3′). RNA isolation and the real-time PCR operation were carried out according to the previously described method (Li et al., 2013). The standard cure method was used to analyze the fold change of M gene mRNA expression level.

Statistical analysis

Statistical analysis was performed using PAWS Statistics 18 (SPSS Inc. USA). Significant differences between NDV-infected RNAi control cells and B23-RNAi cells, and between B23 or its mutants overexpression and controls in viral $TCID_{50}$ measurement, HA test and in mRNA expression levels of NDV M gene was analyzed by the Independent-Samples *T*-test. Values of $P < 0.05$ (∗) are considered significant.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31172338), a project funded by the Excellent Doctoral Dissertation of Yangzhou University, the Earmarked Fund for Modern Agro-industry Technology Research System (nycytx-41-G07), the Priority Academic Program Development of Jiangsu Higher Education Institutions and the Chinese Special Fund for Agro-scientific Research in the public interest (201303033).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.virol.2014.01.011.

References

Adachi, Y., Copeland, T.D., Hatanaka, M., Oroszlan, S., 1993. Nucleolar targeting signal of Rex protein of human T-cell leukemia virus type I specifically binds to nucleolar shuttle protein B-23. J. Biol. Chem 268 (19), 13930–13934.
Alexander, D.J., 2000. Newcastle disease and other avian paramyxoviruses. Rev. Sci. Tech. 19 (2), 443–462.
Andersen, J.S., Lam, Y.W., Leung, A.K., Ong, S.E., Lyon, C.E., Lamond, A.I., Mann, M., 2005. Nucleolar proteome dynamics. Nature 433 (7021), 77–83.
Battisti, A.J., Meng, G., Winkler, D.C., McGinnes, L.W., Plevka, P., Steven, A.C., Morrison, T.G., Rossmann, M.G., 2012. Structure and assembly of a paramyxovirus matrix protein. Proc. Natl. Acad. Sci. U. S. A. 109 (35), 13996–14000.
Boisvert, F.M., van Koningsbruggen, S., Navascues, J., Lamond, A.I., 2007. The multifunctional nucleolus. Nat. Rev. Mol. Cell Biol. 8 (7), 574–585.
Coleman, N.A., Peeples, M.E., 1993. The matrix protein of Newcastle disease virus localizes to the nucleus via a bipartite nuclear localization signal. Virology 195 (2), 596–607.
Duan, Z.Q., Chen, J., He, L., Xu, H.X., Li, Q.H., Hu, S.L., Liu, X.F., 2013a. Matrix protein of Newcastle disease virus interacts with avian nucleophosmin B23.1 in HEK-293T cells. Wei. Sheng. Wu. Xue. Bao. 53 (7), 730–736.
Duan, Z., Li, Q., He, L., Zhao, G., Chen, J., Hu, S., Liu, X., 2013b. Application of green fluorescent protein-labeled assay for the study of subcellular localization of Newcastle disease virus matrix protein. J. Virol. Methods 194 (1-2), 118–122.
Emmott, E., Dove, B.K., Howell, G., Chappell, L.A., Reed, M.L., Boyne, J.R., You, J.H., Brooks, G., Whitehouse, A., Hiscox, J.A., 2008. Viral nucleolar localisation signals determine dynamic trafficking within the nucleolus. Virology 380 (2), 191–202.
Emmott, E., Hiscox, J.A., 2009. Nucleolar targeting: the hub of the matter. EMBO Rep. 10 (3), 231–238.
Eulalio, A., Nunes-Correia, I., Carvalho, A.L., Faro, C., Citovsky, V., Salas, J., Salas, M.L., Simoes, S., de Lima, M.C., 2006. Nuclear export of African swine fever virus p37 protein occurs through two distinct pathways and is mediated by three independent signals. J. Virol. 80 (3), 1393–1404.
Fankhauser, C., Izaurralde, E., Adachi, Y., Wingfield, P., Laemmli, U.K., 1991. Specific complex of human immunodeficiency virus type 1 rev and nucleolar B23 proteins: dissociation by the Rev response element. Mol. Cell Biol. 11 (5), 2567–2575.
Ghildyal, R., Baulch-Brown, C., Mills, J., Meanger, J., 2003. The matrix protein of Human respiratory syncytial virus localises to the nucleus of infected cells and inhibits transcription. Arch.Virol. 148 (7), 1419–1429.
Greco, A., 2009. Involvement of the nucleolus in replication of human viruses. Rev. Med. Virol. 19 (4), 201–214.
Harrison, M.S., Sakaguchi, T., Schmitt, A.P., 2010. Paramyxovirus assembly and budding: building particles that transmit infections. Int. J. Biochem. Cell Biol. 42 (9), 1416–1429.
Hingorani, K., Szebeni, A., Olson, M.O., 2000. Mapping the functional domains of nucleolar protein B23. J. Biol. Chem. 275 (32), 24451–24457.
Hiscox, J.A., 2002. The nucleolus – a gateway to viral infection? Arch. Virol. 147 (6), 1077–1089.
Hiscox, J.A., 2007. RNA viruses: hijacking the dynamic nucleolus. Nat. Rev. Microbiol. 5 (2), 119–127.
Hiscox, J.A., Whitehouse, A., Matthews, D.A., 2010. Nucleolar proteomics and viral infection. Proteomics 10 (22), 4077–4086.
Huang, N., Negi, S., Szebeni, A., Olson, M.O., 2005. Protein NPM3 interacts with the multifunctional nucleolar protein B23/nucleophosmin and inhibits ribosome biogenesis. J. Biol. Chem. 280 (7), 5496–5502.
Huang, W.H., Yung, B.Y., Syu, W.J., Lee, Y.H., 2001. The nucleolar phosphoprotein B23 interacts with hepatitis delta antigens and modulates the hepatitis delta virus RNA replication. J. Biol. Chem. 276 (27), 25166–25175.
Itahana, K., Bhat, K.P., Jin, A., Itahana, Y., Hawke, D., Kobayashi, R., Zhang, Y., 2003. Tumor suppressor ARF degrades B23, a nucleolar protein involved in ribosome biogenesis and cell proliferation. Mol. Cell. 12 (5), 1151–1164.
Iwasaki, M., Takeda, M., Shirogane, Y., Nakatsu, Y., Nakamura, T., Yanagi, Y., 2009. The matrix protein of measles virus regulates viral RNA synthesis and assembly by interacting with the nucleocapsid protein. J. Virol. 83 (20), 10374–10383.

Lechertier, T., Sirri, V., Hernandez-Verdun, D., Roussel, P., 2007. A B23-interacting sequence as a tool to visualize protein interactions in a cellular context. J. Cell. Sci. 120 (Pt 2), 265–275.
Lee, C., Hodgins, D., Calvert, J.G., Welch, S.K., Jolie, R., Yoo, D., 2006. Mutations within the nuclear localization signal of the porcine reproductive and respiratory syndrome virus nucleocapsid protein attenuate virus replication. Virology 346 (1), 238–250.
Li, X., Cao, H., Wang, Y., Zheng, S.J., 2013. Engagement of new castle disease virus (NDV) matrix (M) protein with charged multivesicular body protein (CHMP) 4 facilitates viral replication. Virus Res. 171 (1), 80–88.
Li, Y.P., 1997. Protein B23 is an important human factor for the nucleolar localization of the human immunodeficiency virus protein Tat. J. Virol. 71 (5), 4098–4102.
Li, Y.P., Busch, R.K., Valdez, B.C., Busch, H., 1996. C23 interacts with B23, a putative nucleolar-localization-signal-binding protein. Eur. J. Biochem. 237 (1), 153–158.
Lindstrom, M.S., 2012. Elucidation of motifs in ribosomal protein S9 that mediate its nucleolar localization and binding to NPM1/nucleophosmin. PLoS One 7 (12), e52476.
Liu, Y.L., Hu, S.L., Zhang, Y.M., Sun, S.J., Romer-Oberdorfer, A., Veits, J., Wu, Y.T., Wan, H.Q., Liu, X.F., 2007. Generation of a velogenic Newcastle disease virus from cDNA and expression of the green fluorescent protein. Arch. Virol. 152 (7), 1241–1249.
Lymberopoulos, M.H., Bourget, A., Ben Abdeljelil, N., Pearson, A., 2011. Involvement of the UL24 protein in herpes simplex virus 1-induced dispersal of B23 and in nuclear egress. Virology 412 (2), 341–348.
Matthews, D.A., 2001. Adenovirus protein V induces redistribution of nucleolin and B23 from nucleolus to cytoplasm. J. Virol 75 (2), 1031–1038.
Mori, Y., Okabayashi, T., Yamashita, T., Zhao, Z., Wakita, T., Yasui, K., Hasebe, F., Tadano, M., Konishi, E., Moriishi, K., Matsuura, Y., 2005. Nuclear localization of Japanese encephalitis virus core protein enhances viral replication. J. Virol. 79 (6), 3448–3458.
Okuwaki, M., Tsujimoto, M., Nagata, K., 2002. The RNA binding activity of a ribosome biogenesis factor, nucleophosmin/B23, is modulated by phosphorylation with a cell cycle-dependent kinase and by association with its subtype. Mol. Biol. Cell 13 (6), 2016–2030.
Oliveira, A.P., Simabuco, F.M., Tamura, R.E., Guerrero, M.C., Ribeiro, P.G., Libermann, T.A., Zerbini, L.F., Ventura, A.M., 2013. Human respiratory syncytial virus N, P and M protein interactions in HEK-293T cells. Virus Res. 177 (1), 108–112.
Pantua, H.D., McGinnes, L.W., Peeples, M.E., Morrison, T.G., 2006. Requirements for the assembly and release of Newcastle disease virus-like particles. J. Virol. 80 (22), 11062–11073.
Peeples, M.E., 1988. Differential detergent treatment allows immunofluorescent localization of the Newcastle disease virus matrix protein within the nucleus of infected cells. Virology 162 (1), 255–259.
Peeples, M.E., Wang, C., Gupta, K.C., Coleman, N., 1992. Nuclear entry and nucleolar localization of the Newcastle disease virus (NDV) matrix protein occur early in infection and do not require other NDV proteins. J. Virol. 66 (5), 3263–3269.
Pinol-Roma, S., 1999. Association of nonribosomal nucleolar proteins in ribonucleoprotein complexes during interphase and mitosis. Mol. Biol. Cell 10 (1), 77–90.
Rajani, K.R., Pettit Kneller, E.L., McKenzie, M.O., Horita, D.A., Chou, J.W., Lyles, D.S., 2012. Complexes of vesicular stomatitis virus matrix protein with host Rae1 and Nup98 involved in inhibition of host transcription. PLoS Pathog. 8 (9), e1002929.
Sirri, V., Urcuqui-Inchima, S., Roussel, P., Hernandez-Verdun, D., 2008. Nucleolus: the fascinating nuclear body. Histochem. Cell Biol. 129 (1), 13–31.
Tsuda, Y., Mori, Y., Abe, T., Yamashita, T., Okamoto, T., Ichimura, T., Moriishi, K., Matsuura, Y., 2006. Nucleolar protein B23 interacts with Japanese encephalitis virus core protein and participates in viral replication. Microbiol. Immunol. 50 (3), 225–234.
Ugai, H., Dobbins, G.C., Wang, M., Le, L.P., Matthews, D.A., Curiel, D.T., 2012. Adenoviral protein V promotes a process of viral assembly through nucleophosmin 1. Virology 432 (2), 283–295.
Valdez, B.C., Perlaky, L., Henning, D., Saijo, Y., Chan, P.K., Busch, H., 1994. Identification of the nuclear and nucleolar localization signals of the protein p120. Interaction with translocation protein B23. J. Biol. Chem. 269 (38), 23776–23783.
Wang, Y., Chen, B., Li, Y., Zhou, D., Chen, S., 2011. PNRC accumulates in the nucleolus by interaction with B23/nucleophosmin via its nucleolar localization sequence. Biochim. Biophys. Acta 1813 (1), 109–119.
Yoshida, T., Nagai, Y., Maeno, K., Iinuma, M., Hamaguchi, M., Matsumoto, T., Nagayoshi, S., Hoshino, M., 1979. Studies on the role of M protein in virus assembly using a ts mutant of HVJ (Sendai virus). Virology 92 (1), 139–154.
Yuan, H., Puckett, S., Lyles, D.S., 2001. Inhibition of host transcription by vesicular stomatitis virus involves a novel mechanism that is independent of phosphorylation of TATA-binding protein (TBP) or association of TBP with TBP-associated factor subunits. J. Virol. 75 (9), 4453–4458.
Yusoff, K., Tan, W.S., 2001. Newcastle disease virus: macromolecules and opportunities. Avian Pathol. 30 (5), 439–455.
Zakaryan, H., Stamminger, T., 2011. Nuclear remodelling during viral infections. Cell Microbiol. 13 (6), 806–813.